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Canadian Journal of Zoology

Réimpression du

Journal canadien de zoologie

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Volume 55 • Number 12 • 1977

Pages 2024–2034

National Research Council Canada

Conseil national de recherches Canada

Pulmonary ventilation: perfusion relationships in terrestrial and aquatic chelonian reptiles

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Received June 27, 1977

BURGGREN, W. W., M. L. GLASS, and K. JOHANSEN, 1977. Pulmonary ventilation: perfusion relationships in terrestrial and aquatic chelonian reptiles. Can. J. Zool. **55**: 2024–2034.

Pulmonary ventilation and perfusion have been measured directly in unanaesthetized turtles (*Pelomedusa subrufa*) and tortoises (*Testudo pardalis*) during normal air breathing and during the inspiration of both hypoxic and hypercapnic gases. Lung ventilation in air was intermittent in both species and particularly in the turtle was accompanied by transient increases in pulmonary perfusion. Hypercapnia (4% CO_2 in air) elicited a twofold to threefold increase in pulmonary perfusion but a sixfold increase in pulmonary ventilation in both species. Consequently the ventilation:perfusion ratio more than doubled in value. Unlike the hypercapnic responses, hypoxia (5% O_2 in N_2) increased pulmonary perfusion by two to five times but increased pulmonary ventilation by less than two times, and so the ventilation:perfusion ratio fell by one-half during hypoxic exposure. These data are interpreted in terms of intermittent breathing and processes of O_2 and CO_2 transport.

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Ce travail a pour objet l'étude de la ventilation pulmonaire et de la perfusion chez les tortues *Pelomedusa subrufa* et *Testudo pardalis*; des tortues non anesthésiées ont donc été soumises à des expériences permettant de mesurer directement ces paramètres à l'air ambiant normal, de même que dans des conditions d'hypoxie et d'hypercapnie. La ventilation pulmonaire à l'air est intermittente chez les deux espèces et s'accompagne, surtout chez *Pelomedusa*, d'augmentations momentanées de la perfusion pulmonaire. La perfusion pulmonaire augmente d'un facteur de deux ou trois dans des conditions d'hypercapnie (4% CO_2 dans l'air) et la ventilation pulmonaire atteint six fois sa valeur normale chez les deux espèces. Le rapport ventilation:perfusion fait plus que doubler dans ces conditions. En revanche, l'hypoxie (5% O_2 dans du N_2) augmente la perfusion pulmonaire d'un facteur de deux à cinq, mais la ventilation pulmonaire de double même pas; le rapport ventilation:perfusion diminue donc de moitié durant l'hypoxie. L'interprétation des résultats s'est faite en fonction de la respiration intermittente et du transport de l'oxygène et du gaz carbonique.

[Traduit par le journal]

Introduction

An organ of gas exchange is in many respects only as effective as is the matching of its ventilation and perfusion. If ventilation: perfusion mismatching occurs, the full potential for gas exchange is not realized, and the metabolic cost of operating the gas exchange organ relative to the O_2 uptake and CO_2 elimination which it achieves becomes excessive and inefficient. Hence, in almost every vertebrate, mechanisms of both a morphological and physiological nature operate to optimize the volumes of air and blood which ventilate and perfuse the lungs. In normal resting birds and mammals ventilation is effectively continuous, i.e. the ventilation frequency, tidal volume, and alveolar (parabronchial) gas composition is static. However, marked fluctuations in ventilation and perfusion may arise from exercise or hypoxic or hypercapnic stresses. Mechanisms such as reflex regulation of heart rate by ventilatory movements serve to match ventilation and perfusion (see Daly (1972) for review), and so play an important role in respiratory gas exchange. In chelonian reptiles, however, lung ventilation is normally a highly intermittent phenomenon. Periods of apnoea of a few seconds to several minutes or even hours in length are punctuated at intervals by either single breaths, as is the case of most tortoises, or by a breathing series composed of many consecutive breaths, as is the case of the aquatic turtles (Shaw and Baldwin 1935; McCutcheon 1943; Lenfant *et al.* 1970; Burggren 1975).

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Effective matching of lung perfusion with lung ventilation in these reptiles obviously demands both acute and continuous cardiovascular adjustments. In the turtle and tortoise these adjustments consist of a redistribution of cardiac output between the pulmonary and systemic circulation, as well as changes in the total level of cardiac output affected by variation in both heart rate and stroke volume (Belkin 1964; White and Ross 1966; Johansen *et al.* 1970; Burggren 1975; Shelton and Burggren 1976).

Previous investigations have demonstrated that the inhalation of hypoxic or hypercapnic gas mixtures may cause considerable alterations in ventilation frequency and tidal volume in reptiles (Randall *et al.* 1944; Atland and Parker 1955; Nielsen 1962; Boyer 1966; Frankell *et al.* 1969; Jackson *et al.* 1974; Glass *et al.* 1977). Much less is known about the effect of inspired or lung gas tensions on cardiovascular performance. Heart rate changes have been reported in reptiles breathing hypoxic or hypercapnic gas mixtures (Boyer 1966; White and Ross 1966; Burggren 1975). Certainly ventilation:perfusion relationships in reptiles have not been quantified even during normal breathing, and the manner in which pulmonary perfusion may be adjusted relative to pulmonary ventilation during respiratory stress is currently completely unknown. Hence, in this study pulmonary ventilation:perfusion relationships during air breathing and during the inspiration of experimental gas mixtures have been investigated in the terrestrial tortoise *Testudo pardalis* and the aquatic turtle *Pelomedusa subrufa* using direct methods and simultaneous and continuous recording of lung ventilation and perfusion.

Materials and Methods

Experiments were performed on four leopard tortoises, *Testudo pardalis*, and four side-necked marsh turtles, *Pelomedusa subrufa*, native to Kenya, East Africa, and airfreighted to the laboratory in Aarhus, Denmark. *Testudo* weighed from 2 to 4 kg, while *Pelomedusa* weighed from 350 to 800 g. The animals had been kept in the laboratory for at least 4 months before experimentation and were healthy and feeding vigorously. All experiments were performed at 25°C on animals in postoperative states.

Blood Pressure and Flow Measurements

Animals anaesthetized with cold torpor (1°C, for 12 h) were restrained ventral side up and a cast cutter was used to excise a piece of the plastron directly over the central blood vessels. The common pulmonary artery and left aorta or left subclavian artery were nonocclusively cannulated in an upstream direction with PE50 polyethylene cannulae. The cannulae were filled with heparinized reptile saline and 200 IU of heparin/kg body weight were injected into each animal at the beginning of the experiment and daily thereafter. The cannulae were connected to Statham 23 Dd pressure transducers whose outputs were displayed on a Brush 260 rectilinear recorder. The overall undamped natural frequency (58 cycles/s) and critical damping (18%) of the pressure recording system in light of a fundamental frequency (heart rate) of less than 0.5 cycles/s resulted in an insignificant phase and amplitude error in the measured wave form. Pressure calibration and zero-level checks were frequently made during the course of each experiment.

Pulmonary arterial blood flow was measured with a Statham 2202 blood flow meter having a nonocculsive zero function. Blood flow transducers were implanted around the left pulmonary artery just distal to the bifurcation of the common pulmonary artery. Flow transducers were calibrated in vitro by allowing saline delivered at a known rate and at a pressure equivalent to mean arterial blood pressure to perfuse an excised piece of pulmonary artery. Stroke volume was determined by integration of blood flow profiles using weighing or square counting methods. Total pulmonary blood flow was assumed to be twice the blood flow measured in the left pulmonary artery.

After surgery was completed, the pressure cannulae and flow transducer leads were guided out through the hole in the carapace and the excised piece of plastron was sealed back into place with rapidly setting epoxy cement. Animals were then allowed to warm to room temperature (25°C).

Measurement of Ventilation Volume

Lung ventilation in unrestrained, unanaesthetized *T. pardalis* complete with implanted pressure cannulae and blood flow transducers was measured by pneumotachographic techniques described elsewhere (Glass *et al.* 1977). Tortoises fitted with a 'face mask air flow transducer' were placed in a large Perspex chamber which allowed some, though not unimpaired, movement. The pressure tubing attached to the air flow transducer, the blood pressure cannulae, and the blood flow transducer leads were guided out through a small hole in the lid of the chamber. The chamber, which was screened from movements of the investigators, was continuously flushed at a rate of 2–3 ℓ/min with either humidified air or gas mixtures of air and N_2, CO_2 and air, or O_2. Gas flow through the chamber did not produce any elevation of chamber pressure. A Searle Medspect II mass spectrometer was used to continuously monitor gas tensions in the chamber, which remained very stable throughout each experiment.

An adaptation of the buoyancy method described by Jackson (1971) was used to monitor ventilation volume in *Pelomedusa* in a small aquarium (see Glass *et al.* 1977). A large funnel flushed continuously with humidified air or experimental gas mixtures was placed directly over the region of the water surface where the turtle lifted its head to breathe.

All animals were given an 8- to 24-h postsurgical period to recover and acclimate to the apparatus. The experimental protocol consisted of monitoring ventilatory and

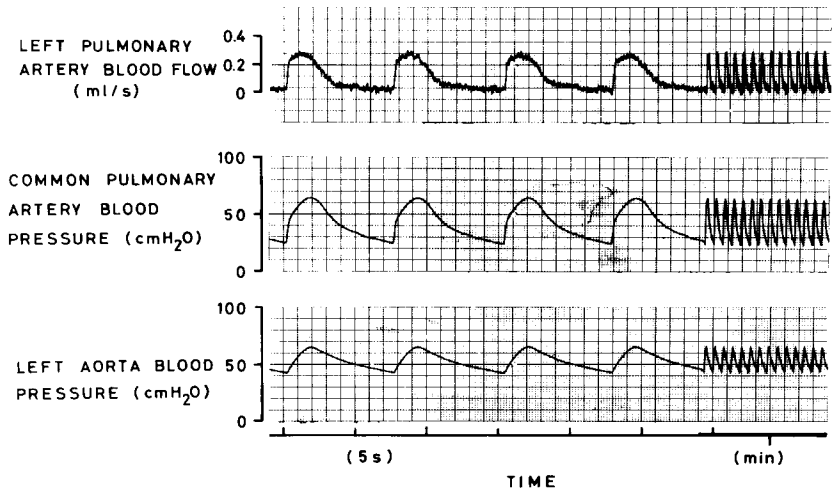

FIG. 1. Representative records of pulmonary blood flow and pressure and systemic blood pressure in an unanaesthetized, unrestrained *Testudo pardalis* during air breathing.

circulatory parameters during air breathing until a complete stabilization of ventilation frequency, tidal volume, heart rate, pulmonary blood flow, and blood pressure was evident. An experimental gas mixture was then introduced into the chamber (tortoises) or underneath the funnel (turtles). Exposure to each experimental gas was continued until a new ventilatory and cardiovascular steady state was achieved. Animals were then permitted to breathe humidified air until ventilatory and circulatory values returned to those levels normally evident during air breathing before the next experimental gas was administered.

End-tidal gas tensions were determined with a Medspect II respiratory mass spectrometer whose sampling catheter was positioned at the nostrils of the animal.

Results

Animals with implanted pressure cannulae and blood flow transducers in which ventilation was also being monitored exhibited very similar patterns of ventilation (i.e. similar frequencies and breath-hold duration) to fully intact, undisturbed animals. Measurements of O_2 consumption on a specimen of *T. pardalis* indicated an O_2 consumption of 20.4 ml/kg per hour before surgery, compared with 20.2 ml/kg per hour measured 12 h after surgery when the animal was in the experimental apparatus. O_2 uptake is often found to be elevated in chelonian reptiles which are under stress, and this further suggests that our experimental animals were not highly disturbed.

Arterial Blood Pressure and Blood Flow

Representative records of systemic and pulmonary arterial blood pressure and left pulmonary arterial blood flow in the tortoise *T. pardalis* are presented in Fig. 1. Systolic blood pressures in the left aorta and in the common pulmonary artery were virtually identical in both *Pelomedusa* and *Testudo* during lung ventilation and during apnoea. Occasionally pulmonary artery systolic pressure was 0.5–1.5 cmH_2O lower than that recorded in the systemic arterial circulation. Systolic pressures in air were about 60–65 cmH_2O in *T. pardalis* and 35–40 cmH_2O in *P. subrufa* (Table 1). Pulmonary pulse pressure was usually twice as large as systemic pulse pressure in both species. Blood flow in the central pulmonary arterial circulation, maximal between pulmonary valve opening and closure, was maintained at significant levels throughout most of diastole, especially during periods of lung ventilation. These data on blood pressure and flow in *T. pardalis* and *P. subrufa* differ from the earlier observations of White and Ross (1966) on aquatic turtles but are very similar to more recently reported measurements of central arterial blood flows and pressures both in conscious, unrestrained and in anaesthetized turtles, *Pseudemys scripta*, and tortoises, *Testudo graeca* (Shelton and Burggren 1976; Burggren 1977).

Intermittent Ventilation and Pulmonary Blood Flow

Lung ventilation in *T. pardalis* consisted of relatively regularly spaced single breaths (frequency, 175 breaths/h) with a tidal volume of about 11 ml/kg (at body temperature and pres-

sure, saturated with water vapor (BTPS)) (Table 1). Pulmonary arterial stroke volume was not normally influenced by these single breaths (Fig. 2A), but activity such as movement about the experimental cage was often accompanied by large increases in pulmonary blood flow. In *P. subrufa* lung ventilation was highly intermittent (frequency, 96 breaths/h) and consisted of several breaths separated by periods of apnoea of variable duration (Fig. 2B). Tidal volume in this species during air breathing was about 33 ml/kg (at BTPS), although large variations were evident as the standard deviations in Table 1 indicate. Changes in pulmonary artery stroke volume very closely reflected lung ventilation in *Pelomedusa*, with increases in stroke volume of 5–10 times often developing with the onset of lung ventilation. Stroke volume in the pulmonary arteries began to decrease when ventilation was teminated, but if two breathing series were separated by less than 0.5 min (Fig. 2B), then pulmonary flow remained high during the brief period of apnoea. Ventilation tachycardia, which is an integral part of the cardiovascular adjustment during intermittent breathing in the turtle *P. scripta* and the tortoise *T. graeca* (White and Ross 1966; Burggren 1975) was not usually evident in *Pelomedusa* and *T. pardalis*.

Ventilation:Perfusion Relationships

Since ventilation in the chelonians is an intermittent process, the ventilation:perfusion ratio of the lungs ($\dot{V}:\dot{Q}$) often falls to zero, even though marked decreases in pulmonary blood flow may develop during apnoea (Fig. 2). Hence, in the short term (i.e. on a breath-to-breath basis) the ratio between lung ventilation and perfusion fluctuates markedly in chelonians, and the value of $\dot{V}:\dot{Q}$ at any particular point in time is much less meaningful than in continuously ventilating vertebrates. The physiological significance of ventilation-correlated changes in pulmonary blood perfusion relates to changing conditions for gas exchange within the lungs and not to ventilation per se. Changes in alveolar gas composition will to some extent reflect lung perfusion changes. Figure 2C shows alveolar(end-tidal) gas composition of the lungs both immediately preceding and during consecutive air breaths in a breathing series. In *Pelomedusa* marked alterations in lung gas composition occur during the course of a breathing series after apnoea. In

TABLE 1. Ventilation and perfusion data in unanaesthetized, undisturbed *Testudo pardalis* and *Pelomedusa subrufa* during breathing of air, 4% CO_2, and 5% O_2

Inspired gas	N	Body weight, g	Heart rate, beats/min	Systemic systolic pressure, cmH_2O	Systemic diastolic pressure, cmH_2O	Pulmonary systolic pressure, cmH_2O	Pulmonary diastolic pressure, cmH_2O	Left pulmonary artery stroke volume, ml/kg	Pulmonary perfusion volume, $ml/kg \cdot h^{-1}$	Tidal volume, ml/kg	Ventilation frequency, breaths/h	Pulmonary ventilation volume, $ml/kg \cdot h^{-1}$	Mean $\dot{V}:\dot{Q}$
Air	4	2872±668	17	62	44	62	23	1.15	729	11	175	1543	2.2
			±8	±9	±7	±9	±11	±0.42	±153	±6	±145	±747	±1.3
4% CO_2	4		18	68	48	67	21	2.75	2504	35	280	9520	5.0
			±11	±19	±17	±20	±5	±1.25	±1622	±24	±136	±5520	±3.2
5% O_2	3		32	70	48	69	26	2.75	4402	17	193	2874	0.9
			±2	±13	±11	±13	±7	±1.18	±2849	±13	±51	±545	±0.8
Air	5	572±234	28	37	28	37	15	0.17	857	33	96	2389	4.7
			±10	±8	±7	±8	±9	±0.21	±1001	±26	±41	±1115	±3.0
4% CO_2	5		32	42	31	41	16	0.28	1504	70	211	13472	10.4
			±10	±10	±9	±10	±6	±0.29	±1304	±30	±94	±5900	±5.6
5% O_2	5		33	36	27	35	12	0.28	1605	35	120	3079	2.6
			±15	±6	±9	±6	±6	±0.23	±1049	±30	±28	±922	±1.6

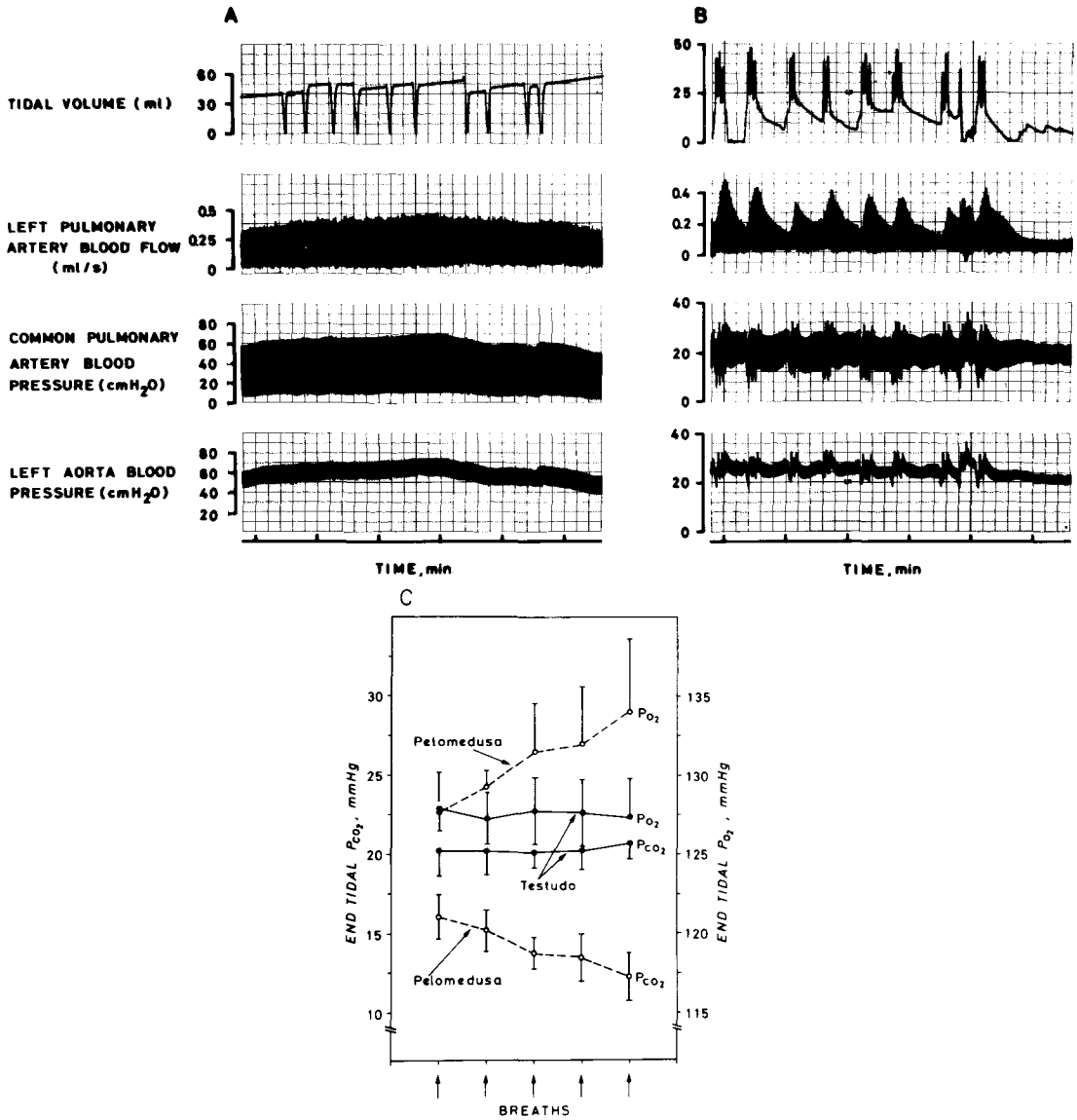

FIG. 2. Lung ventilation and perfusion in (A) *Testudo pardalis* and (B) *Pelomedusa subrufa* during voluntary air breathing. Time marker in minutes. (C) Illustration of changes in end-tidal P_{O_2} and P_{CO_2} during a breathing series of six breaths in *Pelomedusa* and during five consecutive breaths in *Testudo*.

Testudo, however, lung gas composition does not change during the brief alternating periods of apnoea and lung ventilation. Hence, at least in *Testudo*, lung gas composition is not particularly expressive of the relationship between pulmonary ventilation and perfusion.

Another means of correlating the changes in ventilation volume and pulmonary blood flow in chelonians practicing such differing patterns of breathing, and a method applicable to both *Testudo* and *Pelomedusa*, is to measure total ventilation and perfusion volumes over comparatively long time periods; in the present study these periods encompassed a minimum of 30 and often 60–80 breaths. From these data both pulmonary ventilation volume ($\dot{V}$) in millilitres air per kilogram body weight per hour and pulmonary perfusion volume ($\dot{Q}$) in millilitres

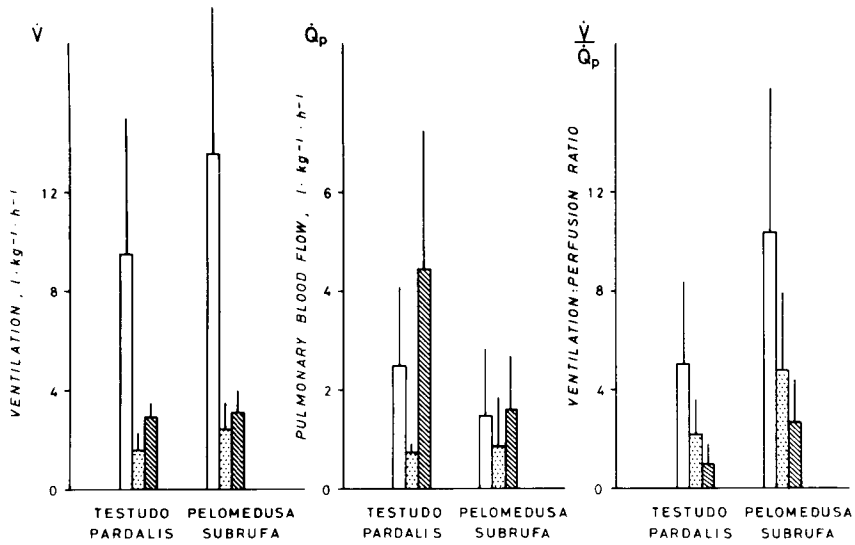

FIG. 3. Ventilation volume, pulmonary perfusion volume, and the $\dot{V}:\dot{Q}$ ratio in *Testudo pardalis* and *Pelomedusa subrufa* during the inspiration of air (dotted bars), 4% CO_2 (open bars), and 5% O_2 (diagonal bars).

blood per kilogram body weight per hour have been calculated for each animal under a variety of experimental conditions, and a $\dot{V}:\dot{Q}$' value determined for the lungs. In this fashion the short-term fluctuations in $\dot{V}:\dot{Q}$ associated with intermittent breathing are instead incorporated into the calculation of a 'mean $\dot{V}:\dot{Q}$' covering a relatively long time period.

Normoxia

Mean values ($\pm$ standard deviations) of respiratory and cardiovascular values derived from these experiments are presented in Table 1. During air breathing $\dot{V}:\dot{Q}$ was 2.2 in *T. pardalis*, while in *P. subrufa* a higher value of 4.7 was evident. However, $\dot{V}:\dot{Q}$ values were not significantly different ($P > 0.10$) in these two species. Heart rate in *Pelomedusa* (28 beats/min) was about double that in *Testudo* (17 beats/min), so although *Pelomedusa* had a much smaller pulmonary stroke volume relative to body weight, pulmonary perfusion volume in *Pelomedusa* was similar to that in *Testudo* during air breathing (Table 1).

Hypercapnia

Both ventilatory and cardiovascular performance changed markedly during the inspiration of hypercapnic gas mixtures. The inspiration of 5% CO_2 proved to be a potent stimulator of ventilation, as Glass *et al.* (1977) have similarly reported for these two chelonians. Respiratory frequency increased two to three times and ventilation increased five to seven times in both *Testudo* and *Pelomedusa* (Table 1). Heart rate was not particularly affected in either species, however, and left pulmonary stroke volume was only doubled, so pulmonary perfusion volume increased two to three times. As a consequence of these alterations in lung ventilation and perfusion, the value of $\dot{V}:\dot{Q}$ increased to 5.0 in *T. pardalis* and 10.4 in *P. subrufa* during the inspiration of 4% CO_2 (Table 1). These changes in $\dot{V}:\dot{Q}$ were significantly different ($P < 0.05$) from air-breathing levels in both species. By means of histograms, Fig. 3 illustrates pulmonary ventilation volume, perfusion volume, and the ratio between these two factors during breathing in air, 4% CO_2, and 5% O_2 in *T. pardalis* and *P. subrufa*. Ventilatory and cardiovascular responses usually developed equally rapidly during hypercapnic breathing in both animals. In *Testudo* the total response often required several minutes to develop, whereas in *Pelomedusa* the full effect was often apparent within 1–2 min, and abated equally rapidly when air breathing was resumed (Fig. 4).

Additional experiments were performed on three *Pelomedusa* in which pulmonary ventilation and perfusion was monitored during the inspiration of air and 2, 4, and 6% CO_2. Ventilation volume and pulmonary perfusion volume were significantly increased above air values ($P <$

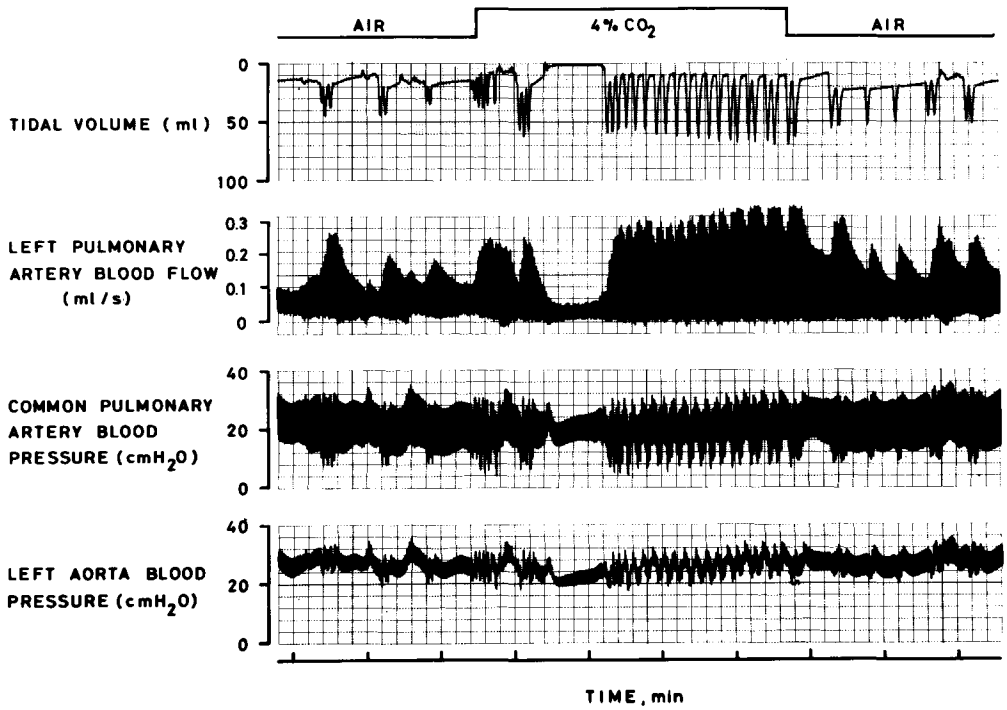

FIG. 4. Records of pulmonary ventilation and perfusion measured in *Pelomedusa subrufa* before, during, and after the inspiration of 4% CO_2.

0.05) during the inspiration of 2% CO_2 and showed the consistently highest values at 6% CO_2 (Fig. 5). $\dot{V}:\dot{Q}$ increased progressively with each increase in CO_2 in inspired gas although changes in $\dot{V}:\dot{Q}$ between air and 6% CO_2 were much more marked in some *Pelomedusa* than in others.

Hypoxia

The inspiration of 5% O_2 affected tidal volume and respiratory frequency to a much smaller extent than did breathing of 4% CO_2, and ventilation increased by less than two times in both species. However, pulmonary perfusion was strongly stimulated by severe hypoxia. Heart rate nearly doubled in *Testudo* and stroke volume increased over two times in both species (Table 1). Hence, pulmonary perfusion volume doubled in *Pelomedusa* and went up over six times in *Testudo*. The net effect was that the $\dot{V}:\dot{Q}$ decreased significantly ($P < 0.05$) to only 0.9 in *T. pardalis* (Fig. 3). The mean $\dot{V}:\dot{Q}$ decreased to 2.6 in *Pelomedusa subrufa*, but this change was not significantly different ($P > 0.10$) from the value evident during air breathing.

Experiments were performed on three *Pelomedusa* in which they were exposed to 5, 10, 21, and 100% O_2. The mean $\dot{V}:\dot{Q}$ in these turtles during the inspiration of hypoxic gas or 100% O_2 was not significantly different ($P > 0.10$) from those in air, although in two of three animals $\dot{V}:\dot{Q}$ fell markedly with decreasing O_2 (Fig. 6). In few turtles, but most tortoises, the inspiration of 100% O_2 was accompanied by a striking reduction in pulmonary stroke volume (Fig. 7).

Discussion

Measurements of pulmonary blood flow and ventilation during intermittent breathing in *Testudo* and *Pelomedusa* have revealed considerable differences in ventilation:perfusion matching. Much greater fluctuations in lung gas tensions in *Pelomedusa* result from often longer and more irregularly spaced periods of apnoea in this species compared with *Testudo*. Changes in end-tidal gas composition and (or) the breathing movements with associated changes in stretch of the lung parenchyma could all be eliciting stimuli for the marked perfusion changes recorded during ventilation in *Pelomedusa* (Figs. 2 and 4, Table 1), although Johansen *et al.* (1977) have shown for another aquatic turtle, *Pseudemys*, that pulmonary

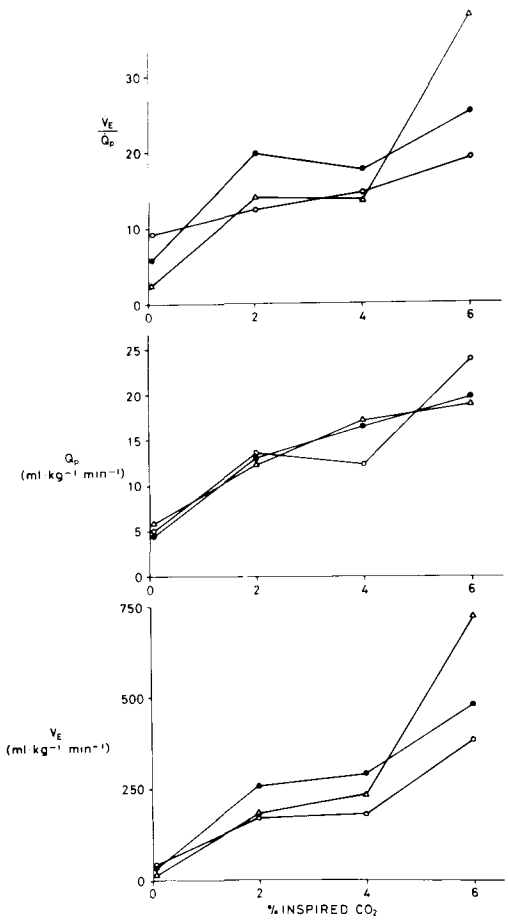

FIG. 5. Ventilation volume, pulmonary perfusion volume, and the $\dot{V}$:$\dot{Q}$ ratio in three *Pelomedusa subrufa* during the inspiration of 0–6% CO_2.

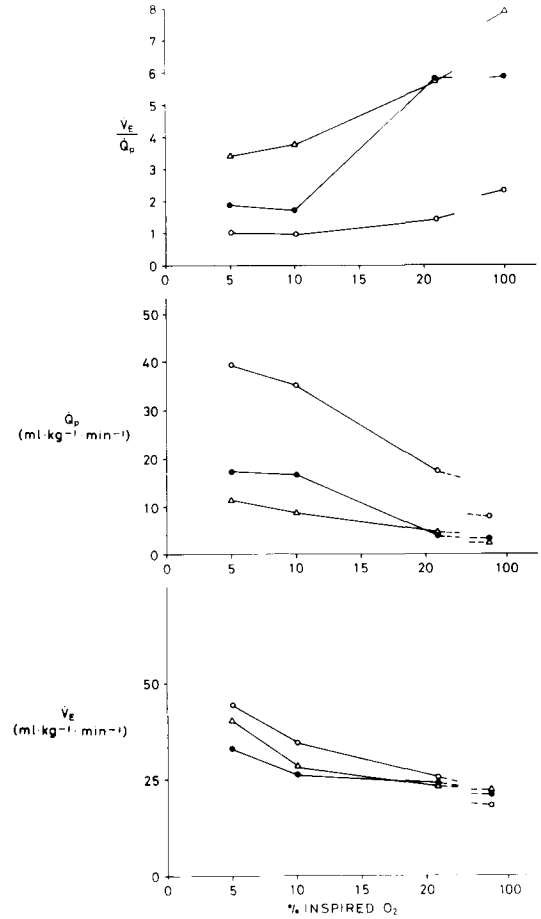

FIG. 6. Ventilation volume, pulmonary perfusion volume, and the $\dot{V}$:$\dot{Q}$ ratio in three *Pelomedusa subrufa* during the inspiration of 5–100% O_2.

stretch receptors are much more important. The greatest blood flow to the lungs of the turtle generally occurs during lung ventilation when alveolar pressure of O_2 ($P_{A_{O_2}}$) is high, alveolar pressure of CO_2 ($P_{A_{CO_2}}$) is low, and so the conditions are the most favourable for respiratory gas transfer between lungs and blood. During apnoea lung perfusion in *Pelomedusa* is curtailed, mainly through a reduction in pulmonary stroke volume (Table 1), and cardiac energy expended in perfusing the lungs is decreased at a time when the potential for O_2 and CO_2 transfer is declining. In *Testudo* breathing is usually much more regular than in *Pelomedusa*, and insignificant changes in lung gas tensions and pulmonary blood flow attend the ventilatory movements (Fig. 2A).

While the pattern of pulmonary perfusion related to ventilation varies between the turtle and tortoise, the $\dot{V}$: $\dot{Q}$ ratio of *Testudo* (2.2) calculated over an extended time period is not markedly different from that of *Pelomedusa* (4.7), nor do the air convection requirements vary widely in these two species (Glass *et al.* 1977). Meaningful comparison of absolute $\dot{V}$: $\dot{Q}$ values between single specimens during air breathing may be complicated by the relatively large variation between individuals in ventilation and (or) perfusion volumes (see standard deviations of Table 1). Since no published information appears to be available on measured or calculated $\dot{V}$: $\dot{Q}$ ratios in chelonians a further comparison with our data is precluded.

Irrespective of any differences in $\dot{V}$: $\dot{Q}$ values during normal air breathing in the turtle and

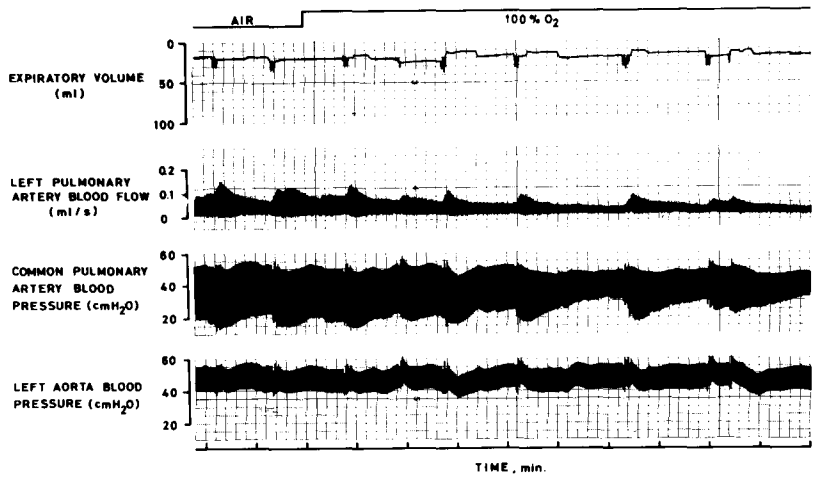

FIG. 7. Ventilation and perfusion parameters measured in *Pelomedusa subrufa* during breathing in air and 100% O_2.

tortoise, largely similar and highly complex physiological adjustments in $\dot{V}:\dot{Q}$ occur in both species during hypoxic and hypercapnic stress. The inhalation of gas containing 2% CO_2 is a potent stimulus for lung ventilation in both *Testudo* and *Pelomedusa* (Fig. 3). Glass *et al.* (1977) have demonstrated that an increase in end-tidal P_{CO_2} by 2–3 mmHg is sufficient to double ventilation volume in these species. Concomittant increases in lung perfusion during hypercapnia are not as great, however, so the $\dot{V}:\dot{Q}$ ratio of the chelonian lungs rises during the inspiration of CO_2. The more severe the hypercapnic stress, the more pronounced is the rise in the pulmonary $\dot{V}:\dot{Q}$, at least in *Pelomedusa* (Fig. 5). Hypoxic stress, on the other hand, results in a marked increase in pulmonary perfusion relative to pulmonary ventilation, and so the $\dot{V}:\dot{Q}$ of the lungs falls significantly, particularly in the tortoise. Unfortunately, simultaneous measurements of blood gases and alveolar gas composition are not available, so an extensive discussion of what actual $\dot{V}:\dot{Q}$ values signify in lung function is precluded. However, the fundamentally different nature of integrated ventilatory and cardiovascular responses to hypoxic and to hypercapnic stress does invite comment about the physiological implications to gas exchange of a change in the $\dot{V}:\dot{Q}$ ratio in *Testudo* and *Pelomedusa*.

As in other vertebrates, oxygen exchange during both normoxia and hypoxia in chelonians is greatly influenced by the S-shaped O_2 dissociation curve of the blood (Lenfant *et al.* 1970; Burggren *et al.* 1977). A significant reduction in inspired P_{O_2} will be accompanied by a reduction in O_2 saturation of blood leaving the lung capillaries. As inspired gas becomes progressively more hypoxic, quite small reductions in inspired P_{O_2} will result in large reductions in percentage blood O_2 saturation, because of the steep slope of the midregion of the blood-O_2 dissociation curve. An increase of pulmonary ventilation volume alone during hypoxia will thus contribute little to an elevation of alveolar P_{O_2}, and hence to an increase in oxygen loading. Moreover, in *Pelomedusa* alveolar P_{O_2}'s during normoxic breathing are very high and reach nearly inspired P_{O_2} values during a normal breath sequence (Fig. 2C). Hyperventilation could do little to reduce the inspired-to-alveolar P_{O_2} gradient in that species. A small inspired-to-alveolar P_{O_2} gradient during normoxic breathing also occurs in *Testudo* and a ventilation increase in excess of the approximate doubling which occurred (Fig. 3) would be ineffective as a compensation during hypoxia. In line with this the inspiration of hypoxic gas does not cause a particularly marked rise in ventilation volume in *Testudo* or *Pelomedusa* (Fig. 3, Glass *et al.* 1977) nor in many other reptiles (see Wood and Lenfant 1976). A compensated transport of O_2 from the lungs to the tissues during hypoxia could more efficiently be achieved if pulmonary blood flow increased and the right-to-left central vascular shunt was kept low. Present data clearly

demonstrate the former of these compensatory measures to occur; the role of the latter cannot be assessed on the evidence available.

The inhalation of 100% O_2 by *Testudo*, and to a lesser extent by *Pelomedusa*, produced a reduction in pulmonary stroke volume with little or no accompanying adjustment in pulmonary ventilation volume (Fig. 7); consequently, $\dot{V}:\dot{Q}$ increased. Although pulmonary blood flow fell, O_2 transfer from the lungs to the blood may not have, for a much greater amount of O_2 will be carried physically in solution in the plasma during 100% O_2 breathing. Whether then the fall in pulmonary blood flow is a response to an increased blood O_2 content or whether this suggests the involvement of a sensory 'hypoxic drive' for maintaining lung perfusion under normoxic conditions is not known.

Processes of pulmonary CO_2 elimination in chelonians are different in many respects from O_2 uptake. CO_2 gradients from pulmonary arterial blood to alveolar gas in *Pelomedusa*, *Testudo*, and other chelonians change little and very slowly with large increases in lung CO_2 (Glass M., W. Burggren, and K. Johansen, unpublished; Burggren 1976). Hence, the amount of CO_2 excreted with each passage of blood through the lungs may differ little during inspiration of both air and CO_2-enriched gas. However, elevated blood and tissue CO_2 levels produce marked changes in pH and bicarbonate and lactate levels, for example. The very pronounced increase in ventilation volume which develops during hypercapnic breathing in *Testudo* and *Pelomedusa* (Fig. 3) may be a reflex-stimulated attempt to lower blood $P_{A_{CO_2}}$ or arterial $P_{A_{CO_2}}$ or raise blood pH by hyperventilation in a fashion similar to that which occurs during hypercapnic stress in homeotherms.

The control mechanisms mediating changes in pulmonary ventilation and perfusion during inspiration of experimental gases are not completely understood. CO_2-sensitive cells occur in the lungs of reptiles (Milsom and Jones 1976), and a direct influence of $P_{A_{CO_2}}$ on lung ventilation, presumably mediated by these cells, has been demonstrated (Gatz *et al.* 1975). Frankel *et al.* (1969), on the other hand, have presented preliminary histological and physiological evidence for arterial chemoreceptors within the carotid arch of the turtle which might influence lung ventilation. Whatever their location, the marked ventilatory responses to hypercapnia compared with hypoxia reported in the present study for unanaesthetized chelonians suggest that CO_2 receptors could potentially exert a large influence over respiratory events during intermittent ventilation, especially in some terrestrial species where aquatic CO_2 elimination is obviated and P_{CO_2} fluctuations may be larger.

Acknowledgements

The authors acknowledge Prof. G. M. O. Maloiy, University of Nairobi, for his generous provision of the animals used in this investigation.

ATLAND, P., and M. PARKER. 1955. Effects of hypoxia upon the box turtle. Am. J. Physiol. **180**: 421–427.

BELKIN, D. A. 1964. Variations in heart rate during voluntary diving in the turtle *Pseudemys concinna*. Copeia, 1964(2): 321–330.

BOYER, D. R. 1966. Comparative effects of hypoxia on respiratory and cardiac functions in reptiles. Physiol. Zool. **39**: 307–316.

BURGGREN, W. W. 1975. A quantitative analysis of ventilation tachycardia and its control in two chelonians *Pseudemys scripta* and *Testudo graeca*. J. Exp. Biol. **63**: 367–380.

——— 1976. An investigation of gas exchange and cardiovascular dynamics in the chelonian reptiles. Ph.D. Thesis, University of East Anglia.

——— 1977. The pulmonary circulation of the chelonian reptile; pharmacology, haemodynamics and morphology. J. Comp. Physiol. B, **116**, 303–323.

BURGGREN, W. W., C. E. W. HAHN, and P. FOËX. 1977. Properties of blood oxygen transport in the turtle *Pseudemys scripta* and the tortoise *Testudo graeca*: effects of temperature CO_2 and pH. Respir. Physiol. **31**: 39–50.

DALY, M. DE B. 1972. Interactions of cardiovascular reflexes. Sci. Basis Med. Annu. Rev. 307–332.

FRANKEL, H. M., A. SPITZER, J. BLAINE, and E. P. SCHOENER. 1969. Respiratory response of turtles (*Pseudemys scripta*) to changes in arterial blood gas composition. Comp. Biochem. Physiol. **31**: 535–546.

GATZ, R. N., M. R. FEDDE, and E. C. CRAWFORD, JR. 1975. Lizard lungs: CO_2 sensitive receptors in *Tupinambis nigrospunctatus*. Experientia, **31**: 455–456.

GLASS, M., W. W. BURGGREN, and K. JOHANSEN. 1977. Ventilation and its control in an aquatic and a terrestrial chelonian reptile. J. Exp. Biol. In press.

JACKSON, D. C. 1971. Ventilatory response to hypoxia in turtles at various temperatures. Respir. Physiol. **18**: 178–187.

JACKSON, D. C., S. E. PALMER, and W. L. MEADOW. 1974. The effects of temperature and carbon dioxide breathing on ventilation and acid–base status of turtles. Respir. Physiol. **20**: 131–146.

JOHANSEN, K., W. W. BURGGREN, and M. GLASS. 1977. Pulmonary stretch receptors regulate heart rate and pulmonary blood flow in the turtle, *Pseudemys scripta*. Comp. Biochem. Physiol. **58**A: 185–191.

JOHANSEN, K., C. LENFANT, and D. HANSON. 1970. Phylogenetic development of pulmonary circulation. Fed. Proc. Fed. Am. Soc. Exp. Biol. **29**(3): 1135–1140.

LENFANT, C., K. JOHANSEN, J. A. PETERSEN, and K. SCHMIDT-NIELSEN. 1970. Respiration in the fresh water turtle, *Chelys fimbriata*. Respir. Physiol. **8**: 261–275.

MCCUTCHEON, F. H. 1943. The respiratory mechanism in turtles. Physiol. Zool. **16**: 255–269.

MILSOM, W. K., and D. R. JONES. 1976. Reptilian pulmonary receptors; mechano- or chemo-sensitive? Nature (London), **261**: 327–328.

NIELSEN, B. 1962. On the regulation of respiration in reptiles II. The effect of hypoxia with and without moderate hypercapnia on the respiration and metabolism of lizards. J. Exp. Biol. **39**(1): 107–117.

RANDALL, W. C., D. E. STULLKEN, and W. A. HIESTAND. 1944. Respiration of reptiles as influenced by the composition of the inspired air. Copeia, 1944(**3**): 136–144.

SHAW, R. J., and F. M. BALDWIN. 1935. The mechanics of respiration in turtles. Copeia, 1935(**1**): 12–15.

SHELTON, G., and W. W. BURGGREN. 1976. Cardiovascular dynamics of the Chelonia during apnoea and lung ventilation. J. Exp. Biol. **64**: 323–343.

WHITE, F. N., and G. ROSS. 1966. Circulatory changes during experimental diving in the turtle. Am. J. Physiol. **211**: 15–18.

WOOD, S. C., and C. LENFANT. 1976. Respiration: mechanics, control and gas exchange. *In* Biology of the Reptilia. Physiology Vol. 5A. *Edited by* C. Gans. Academic Press, New York.