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MECHANICAL ANALYSIS OF SPONTANEOUS BREATHING IN THE SEMI-AQUATIC TURTLE, *PSEUDEMYS SCRIPTA*

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SUMMARY

The normal breathing pattern of *Pseudemys scripta* (Schoepff) consists of a continuous burst of breaths separated by a variable period of breath holding. Under normoxic conditions, tidal volume was $6{\cdot}9\,ml\,kg^{-1}$ and the number of breaths was $1{\cdot}9\,min^{-1}$. Increases in pulmonary ventilation upon stimulation by hypercapnia (3 % CO_2) or hypoxia (4 % O_2) are caused primarily by increases in the number of breaths per minute due to a shortening of the breath-hold period. Tidal volume and breath duration remain unchanged. The instantaneous breathing frequency ($f' = 60/T_{tot}$) of $35 \pm 2\,min^{-1}$ corresponds to continuous pump frequencies that minimize the rate of the mechanical work of breathing in anaesthetized turtles. This indicates that turtles breathe at a combination of tidal volume and f' that minimizes the power required to ventilate the lungs. To increase ventilation, the breath hold is shortened and more breaths are taken at this optimal combination. Bilateral vagotomy drastically alters the breathing pattern, producing an elevation in tidal volume, a slowing of breathing frequency, and a prolongation of breath duration while total ventilation remains unchanged. These data suggest that periodic breathing in this species may represent an adaptive strategy which is under vagal control and which serves to minimize the cost of breathing.

INTRODUCTION

In the anaesthetized semi-aquatic turtle, *Pseudemys scripta*, there is a specific combination of pump volume and pump frequency for which minimum power is required to maintain any given level of total ventilation (Vitalis & Milsom, 1986). These results are consistent with those obtained from mammals and lizards, despite great differences in dynamic pulmonary mechanics between the various species (Crosfill & Widdicombe, 1961; Milsom & Vitalis, 1984; Vitalis & Milsom, 1986).

One assumption inherent in such analyses is that breathing is continuous. Due to their low metabolic rates, however, poikilotherms do not normally breathe continuously, but instead exhibit various patterns of periodic breathing. These patterns range from one of single breaths separated by variable periods of breath holding at

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end-inspiration, as seen in the Tokay gecko, to one consisting of bursts of continuous breathing separated by variable periods of breath holding, as seen in semi-aquatic turtles (McCutcheon, 1943; Gans & Hughes, 1967; Shelton, Jones & Milsom, 1985).

For animals taking single breaths, it has been suggested that the work required to take each breath is a more meaningful criterion for assessing the mechanical efficiency of spontaneous breathing than minute work (power) which is used for animals that breathe continuously (Milsom, 1984). Using such an analysis, it was postulated that periodic breathing in the Tokay gecko may represent an adaptive strategy to minimize the cost of breathing.

The pattern of burst breathing exhibited by *Pseudemys scripta* lies between the continuous breathing pattern of mammals and the single breath pattern of lizards. In the present paper, the spontaneous breathing pattern found in this turtle, and the ventilatory responses it exhibits to changes in respiratory drive, are analysed in terms of the work required to take single breaths and the power required for continuous breathing. The results of this analysis are used to test the hypothesis that the periodic breathing patterns seen in poikilotherms tend to minimize the cost of ventilation.

MATERIALS AND METHODS

Twelve turtles [*Pseudemys scripta*, 536 ± 40·9 g mean (± S.E.M.) body weight] were used. The animals were housed in a large circular tank (1·5 m diameter) supplied with flowing tap water (10–15 °C) to a depth of 30 cm. Dry areas heated with lamps were provided to allow the animals to bask. Several days before experiments the animals were moved into the laboratory and housed in smaller tanks (45×60 cm) supplied with water and dry basking areas where they became acclimated to room temperature (20–22 °C). All animals were in a post-absorptive state during experiments.

Measurements of ventilation

At the beginning of each experiment, a turtle was transferred to a tank, with a surface area of 900 cm^2. The tank was covered just below the water level with a grid containing a 10-cm diameter breathing hole. This hole was covered with a ventilation chamber as described by Glass, Boutilier & Heisler (1983) and, as in that study, ventilation was measured by pneumotachography using a Fleish model 00 pneumotachograph, Validyne DP 103-18 differential pressure transducer and Gould integrating amplifier. The system was calibrated by injecting known volumes of air into the chamber at the rate of active ventilation of the animal. Due to the large surface area of the tank, a 10-ml breath would cause a 0·01-cm change in the water level of the tank. Each animal was allowed 12–24 h to adjust to this set-up before any measurements were made.

Ventilation was first measured while animals breathed air and then during random administrations of 4% O_2 in N_2 (hypoxia), and 3–5% CO_2 in air (hypercapnia). Gases were mixed using calibrated flow meters and delivered at a constant rate of

500 ml min^{-1}. Inlet gas composition was checked periodically with a Beckman OM-11 oxygen analyser and an LB-2 CO_2 analyser calibrated with commercially purchased, analysed, gas mixtures. The zero balance of the pneumotachograph was adjusted to cancel the constant signal resulting from gas flow through the system. Animals breathed each gas mixture for 1 h and then ventilation was recorded for 1 h. Afterwards the animals were returned to breathing air for 1 h before the administration of the next gas, as described above. All measurements were made during daytime (08.00–20.00 h) and are taken to represent steady-state conditions in animals equilibrated to the various gas mixtures.

Bilateral vagotomy

Turtles were mildly anaesthetized with sodium pentobarbital (20 mg kg^{-1}, intraperitoneally) and restrained in the prone position. A local anaesthetic (lidocaine) was then administered to the neck and an incision made along one side of the neck. The carotid artery was exposed, the vagus nerve was carefully freed from the carotid artery and approximately 5 mm of the nerve was removed. The incision was then closed and the procedure was repeated on the other side. The animals were allowed to recover for several days and only those animals that were active, showed good reflexes, and were free of infection were used for experiments. These animals were then subjected to the same protocol for the measurement of ventilation as described above.

Calculation of ventilation

All calculations of minute ventilation were based on inspired volume. Breathing frequency, f, (breaths min^{-1}) was determined by dividing the total number of breaths by the total length of the recording, which was usually 1 h or more. Average values for tidal volume (V_T) and breath length (T_{tot}) were determined for each animal on each gas by analysing 15–20 randomly chosen breaths. Total ventilation was calculated as the product of tidal volume, V_T, (ml) and breathing frequency. The instantaneous breathing frequency (f′) was calculated as the total time required to take a single breath, T_{tot}, (s) divided into 60 s.

Measurement of dead space volume (V_D)

The anatomical dead space of the respiratory system was determined for six animals with a mean weight of 820 ± 82 g. Anatomical dead space was considered to be the volume of the trachea and primary bronchi from the glottis to the lung hilus and did not include the volume of the intrapulmonary primary bronchi. Two methods were used to determine V_D. One method involved measuring the volume of the trachea by filling it with water after it had been dissected from the animal. The other method involved measuring the length of the trachea and its diameter, from which its volume was calculated. These measurements are considered to be minimum values for V_D, since they did not include any adjustment for physiological dead space. They represent the volume of gas which must be moved before fresh gas reaches the gas exchange surfaces.

Effect of changing ventilation frequency at constant minute ventilation ($\dot{V}_I$) on blood gas partial pressure

These experiments were performed on six turtles (mean weight 753 ± 81 g) to determine the effect of increasing ventilation (pump) frequency, with concomitant decrease in tidal (pump) volume to maintain total air flow ($\dot{V}_I$) constant, on alveolar ventilation ($\dot{V}_A$, ml min^{-1}) and on arterial P_{O_2} and P_{CO_2}. Each animal was anaesthetized with an injection of sodium pentabarbital (20 mg kg^{-1}, intraperitoneally) and a midline incision was made along the neck of the animal and the carotid artery was exposed. The artery was cannulated with PE 50 tubing filled with heparinized saline. The cannula was fed several centimetres down the artery towards the heart to ensure that the blood sampled was representative of arterial blood leaving the heart. The Pa_{O_2} and Pa_{CO_2} of this blood were measured using Radiometer electrodes and a Radiometer PHM 71 blood gas analyser operated at room temperature.

The trachea was cannulated and the cannula attached to a Harvard Small Animal Respirator (Model 665) with a pneumotachograph in the air flow line to monitor air flow and pump volume. The animals were ventilated at a constant rate of 50 ml min^{-1} kg^{-1} for 15 min. Ventilation began at 10 cycles min^{-1} and frequency was increased in steps of 10–60 cycles min^{-1} accompanied by the appropriate reductions in pump volume to keep total ventilation constant at 50 ml min^{-1} kg^{-1}. Small quantities of arterial blood were removed and analysed for P_{O_2} and P_{CO_2} after each 15-min interval of ventilation. The sample was returned to the animal after each measurement. The pump was returned to 10 cycles min^{-1} after the final measurement and arterial blood was again analysed to ensure that the blood gases returned to their original levels. If this did not occur the data were discarded.

Mean values are given ±1 S.E.M., except where otherwise stated.

RESULTS

The respiratory pattern of *Pseudemys scripta* breathing air, under normal resting conditions, consists of episodes of continuous breathing separated by breath-hold periods of variable duration which are commonly associated with diving. This is illustrated in Fig. 1A, which shows a sample trace of respiratory air flow from an intact animal. From such traces the respiratory variables were calculated and are shown in Table 1. When respiration was stimulated by hypoxic and hypercapnic gas mixtures, total ventilation increased by 1·3 and 2·1 times, respectively. The increase in total ventilation was due solely to an increase in the number of breaths per minute (f), with tidal volume remaining relatively unchanged. This increase in f was not due to an increase in instantaneous frequency (f′), but solely due to the animal taking more breaths at a relatively fixed rate and depth, thus shortening the periods of breath holding (TNVP). The work/breath (W/b) values shown in this table were taken from fig. 6 in Vitalis & Milsom (1986) using the spontaneous values for f′ and VT measured in the present study. Work per minute ($\dot{W}$) is simply the product of W/b and f. Since VT and f′ remained relatively unchanged during respiratory

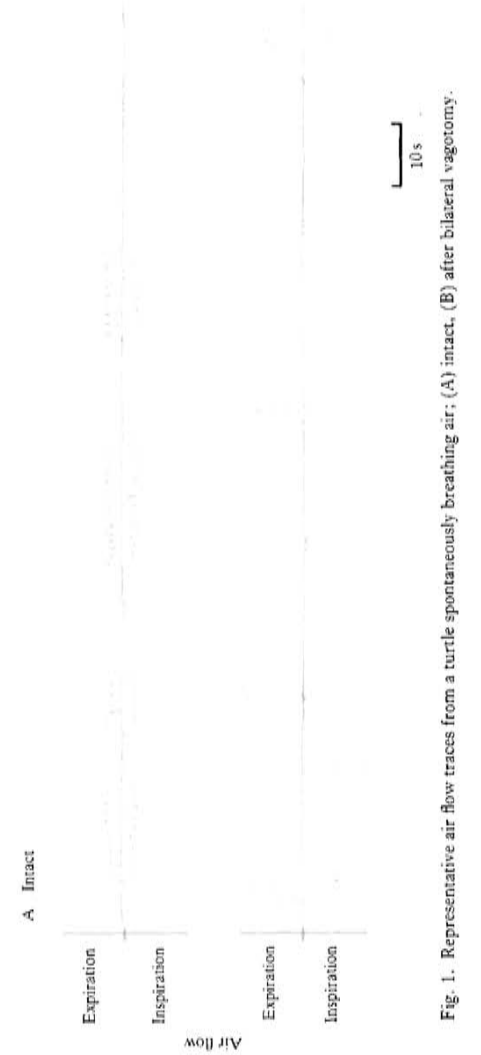

Fig. 1. Representative air flow traces from a turtle spontaneously breathing air; (A) intact, (B) after bilateral vagotomy.

stimulation, W/b also remained unchanged. However, $\dot{W}$ increased when respiration was stimulated by hypoxia or hypercapnia because of increases in f.

The effects of bilateral vagotomy on respiratory air flow are shown in Fig. 1B, and the mean values of the respiratory variables measured under these conditions are also listed in Table 1. During air breathing, vagotomy results in large increases in V_T and a reduction in f and f′, with total ventilation being slightly reduced when compared to values recorded in intact animals. The breathing pattern now consists of single breaths rather than bursts of continuous breathing, although this pattern was not seen in all animals.

Under conditions of hypoxia and hypercapnia, total ventilation after vagotomy increased 2·6-fold and 4·2-fold, respectively. The increase in ventilation stimulated by hypoxia was primarily due to a twofold increase in V_T, and a very small increase in frequency. There was also a lengthening of each breath so that f′ was further reduced to 21 min^{-1}. The increase in $\dot{V}_I$ under hypercapnic conditions was due to a 1·6-fold rise in V_T accompanied by a 2·7-fold increase in breathing frequency (f), with instantaneous frequency (f′) falling further to 21 min^{-1}.

As a consequence of the large increases in V_T and reductions in f′, the calculated W/b in vagotomized animals increased dramatically compared with the values calculated for intact animals under all conditions. During air breathing, W/b in the vagotomized condition increased fivefold compared with values calculated for the intact animals, but because of the decrease in f, $\dot{W}$ rose only 1·8-fold per unit

Table 1. *Respiratory variables for spontaneously ventilating, intact and vagotomized turtles breathing air, 3–5% CO_2 in air and 4% O_2 in N_2*

		Air	3–5 % CO_2	4 % O_2
Intact turtles				
V_T	(ml kg^{-1})	6·9 ± 1·2	6·8 ± 0·4	6·2 ± 0·8
f	(breaths min^{-1})	2·0 ± 0·7	4·2 ± 1·3	3·0 ± 0·4
$\dot{V}_I$	(ml kg^{-1} min^{-1})	13·8	28·3	18·6
f′	(min^{-1})	35 ± 2	34 ± 2	35 ± 2
W/b	(ml cmH_2O kg^{-1})	23	22	19
$\dot{W}$	(ml cmH_2O min^{-1} kg^{-1})	46	92	57
Vagotomized turtles				
V_T	(ml kg^{-1})	18·0 ± 4·0	28·0 ± 6·0	34·2 ± 6·0
f	(breaths min^{-1})	0·6 ± 0·2	1·6 ± 0·9	0·8 ± 0·2
$\dot{V}_I$	(ml kg^{-1} min^{-1})	10·8	45·4	27·7
f′	(min^{-1})	25 ± 3	19 ± 3	21 ± 2
W/b	(ml cmH_2O kg^{-1})	110	220	340
$\dot{W}$	(ml cmH_2O min^{-1} kg^{-1})	66	352	272

Tidal volume (V_T), breathing frequency (f) and instantaneous frequency ($f' = 60/T_{tot}$) are expressed as means ± S.E.M. for six animals.

Total minute ventilation ($\dot{V}_I$) is the product of V_T and f. The work per breath (W/b) values are calculated from Vitalis & Milsom (1986), using spontaneous values for V_T and f′.

Minute work ($\dot{W}$) is the product of W/b and f.

ventilation. Upon exposure to hypoxia and hypercapnia, W/b increased four and three times, respectively, compared with values obtained for the air-breathing condition. The increases in $\dot{W}$ under hypoxic and hypercapnic conditions in the vagotomized animals gave values which were 3·2 and 2·4 times greater, respectively, than similar values calculated for intact animals.

The values obtained for the anatomical dead space of the respiratory system (V_D) of these turtles are shown in Table 2. The mean value of V_D, calculated from measurements of tracheal and bronchial length and diameter, was $0{\cdot}65 \pm 0{\cdot}06$ ml kg^{-1}. Measurements of V_D based on water displacement yielded a mean value of $0{\cdot}74 \pm 0{\cdot}06$ ml kg^{-1}. The difference between the two values was insignificant (Table 2). Since the volume of the trachea is a minimum value for V_D and does not take into account the volume of the intrapulmonary bronchi or physiological dead space, the larger value of 0·74 ml kg^{-1} was used to calculate $\dot{V}_A$.

In the previous paper (Vitalis & Milsom, 1986), the effects of changes in ventilation frequency and volume on the work of breathing were considered in terms of their effect on total ventilation ($\dot{V}_I$ or $\dot{V}_P$). In terms of gas exchange in a living animal, however, changes in alveolar minute ventilation ($\dot{V}_A$) are of more concern than changes in total ventilation. $\dot{V}_A$ is the product of the alveolar ventilation volume ($V_A = V_T - $ dead space, V_D) and ventilation frequency (f). As f increases at a constant level of $\dot{V}_I$, V_T must decrease. Since the dead space volume of the respiratory system is fixed anatomically, as V_T decreases, $\dot{V}_A$ becomes disproportionately small, and when $V_T = V_D$, $\dot{V}_A$ will equal zero. At this point, although much gas is still being moved in and out of the trachea, there is no gas being turned over in the lungs. As a consequence, there are limits to the extent to which V_T may be reduced before gas exchange is compromised by the fall in $\dot{V}_A$. This is illustrated in Fig. 2, which shows the effects of decreasing $\dot{V}_A$, at a constant $\dot{V}_I$ of 50 ml min^{-1} kg^{-1}, on Pa_{O_2} and Pa_{CO_2}. $\dot{V}_A$ values were calculated using a V_D of 0·74 ml kg^{-1} from Table 2. As $\dot{V}_A$ falls with increasing f, arterial P_{O_2} levels also

Table 2. *Values of anatomical dead space determined for specimens of* Pseudemys scripta *by two different methods*

		Dead space	
	Mass (g)	A (ml kg^{-1})*	B (ml kg^{-1})†
	729	0·74	—
	680	0·66	0·63
	755	0·42	—
	1269	0·63	0·95
	725	0·55	0·65
	759	0·88	0·72
$\bar{x}$	820	0·65	0·74
S.E.M.	82·7	0·058	0·063

* Calculated from measurements of the length and diameter of the trachea.

† Determined by water displacement. The difference in values obtained by the two methods of volume determination is insignificant ($\Delta = 0{\cdot}09 \pm 0{\cdot}1537$) at the 95% confidence limit.

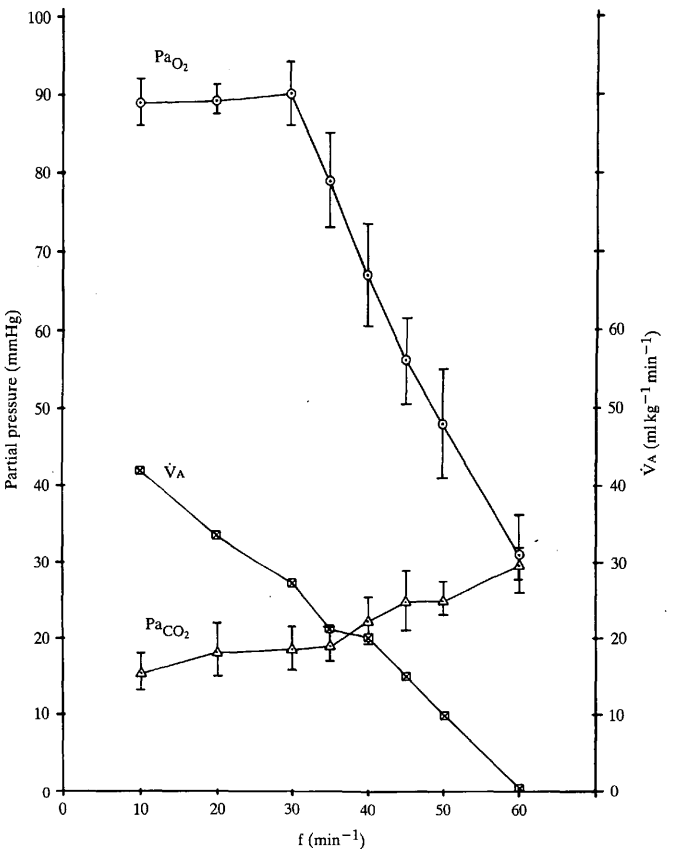

Fig. 2. The effect of increasing ventilation frequency (f) at a constant minute ventilation of 50 ml min^{-1} kg^{-1} on Pa_{O_2} (○), Pa_{CO_2} (△) and alveolar minute ventilation $\dot{V}_A$ (□). The values for Pa_{O_2} and Pa_{CO_2} are the means for six animals ± S.E.M. $\dot{V}_A$ was calculated using a value of 0·74 ml kg^{-1} ± 0·06 for dead space volume (V_D).

steadily decrease from 90 mmHg at an f value of 30 cycles min^{-1} to 30 mmHg at an f value of 60 cycles min^{-1}. Arterial P_{CO_2} steadily rises from 15 to 29 mmHg over the range of frequencies used.

In the present experiments, the fall in $\dot{V}_A$ is imposed as a series of step changes causing an unsteady state where Pa_{O_2} and Pa_{CO_2} are changing to new steady values. The new values of Pa_{O_2} and Pa_{CO_2} will depend on the oxygen consumption and carbon dioxide production of the tissues and the blood gas stores in the venous system. The time course of the equilibration will depend on the capacitance and

conductance of the entire system. As $\dot{V}_A$ approaches zero, the system will increasingly move towards a disequilibrium as more oxygen is continually removed from the venous stores but not replenished. At zero $\dot{V}_A$, arterial blood gas levels will be determined by venous levels and P_{O_2} will continue to fall as oxidative metabolism continues. While it is unclear from the results of these experiments whether steady states are achieved with each step change in ventilation, the dramatic fall in oxygen and rise in carbon dioxide show the profound effect of increasing frequency and decreasing tidal volume, while total ventilation is held constant, on gas exchange and blood gas levels.

DISCUSSION

The breathing patterns recorded in resting, spontaneously breathing *Pseudemys scripta* in the present study are similar to those previously recorded in this and other semi-aquatic turtles (see Glass & Wood, 1983; Shelton *et al.* 1985 for reviews) (Table 3). The values recorded for f (breaths min^{-1}) fall in the upper range of recorded values for semi-aquatic turtles, whereas values of V_T (ml kg^{-1}) recorded for *P. scripta* in this study are at the lower end of previously reported values.

The increases measured in ventilation during exposure to hypercapnic and hypoxic gases in the present study were due solely to an increase in f with V_T, breath duration (T_{tot}) – and therefore f′ – remaining unchanged. These changes are in general agreement with results from other studies (Jackson, 1973; Benchetrit & Dejours, 1980; Milsom & Jones, 1980; Glass *et al.* 1983), with one notable exception. The responses to hypercapnia reported in the literature generally include an elevation of V_T as well as an increase in f (Table 3). This discrepancy may be due to differences in methodology or to a lack of equilibrium between blood gases and inspired gas in the present study, since blood gas values were not determined to confirm that equilibrium had been reached.

The general picture to emerge is that although semi-aquatic turtles may (hypercapnia) or may not (hypoxia) increase V_T in response to respiratory stimuli, they increase respiratory frequency solely by shortening the breath-hold period and increasing the number of breaths per minute. The length of each breath (T_{tot}), and hence the instantaneous breathing frequency (f′), remain unchanged.

Semi-aquatic turtles such as *P. scripta* exhibit episodic or burst breathing whether in water or on land. Whatever combinations of respiratory variables initiate and terminate ventilatory periods, the longer the breath-hold interval, the greater the level of ventilation required to replenish oxygen stores in the lung and blood and to eliminate carbon dioxide when breathing resumes. During the bouts of continuous breathing, the work required to ventilate the lungs can be analysed, as in mammals, in terms of the minute work ($\dot{W}$) or power required to sustain that level of ventilation.

In our previous paper, measurements made on continuously pump-ventilated anaesthetized animals indicated that the power required to maintain a constant level of total ventilation ($\dot{V}_I$) was least at pump frequencies between 35 and 45 breaths min^{-1}

Table 3. *Comparison of respiratory variables for semi-aquatic turtles taken from the literature*

ecies	Temperature (°C)	Respiratory gas	V_T ($ml\,kg^{-1}$)	f (min^{-1})	$\dot{V}$ ($ml\,min^{-1}kg^{-1}$)	Source
eudemys floridana	20	Air	12·6	1·4	18·6	Kinney & White (1977)
eudemys scripta	20	Air	6·9 ± 1·2	2·0 ± 0·07	13·8	Present study
eudemys scripta	20	Air	15·7 ± 2·1	1·6 ± 0·2	23·8 ± 3·4	Jackson, Palmer & Meadow (1974)
eudemys scripta	20	Air	8·8*	1·4 ± 0·15	12·3 ± 1·3	Jackson (1973)
rysemys picta	20	Air	10·7 ± 1·2	1·9 ± 0·27	20·3	Glass, Boutilier & Heisler (1983)
rysemys picta	22–25	Air	13·5 ± 0·9	1·8 ± 0·2	24·8 ± 3·4	Milsom & Jones (1980)
studo horsfieldi	23–25	Air	8·1	1·4	11·5	Benchetrit & Dejours (1980)
		Hypoxia				
eudemys scripta	20	3% O_2	6·8*	2·58 ± 0·25	17·7	Jackson (1973)
eudemys scripta	20	4% O_2	6·2 ± 0·8	3·0 ± 0·4	18·6	Present study
rysemys picta	20	5% O_2	18·7	2·16	30·0	Glass *et al.* (1983)
		Hypercapnia				
eudemys scripta	20	4% CO_2	29·4 ± 3·6	3·0 ± 0·4	84·4 ± 13·3	Jackson *et al.* (1974)
rysemys picta	22–25	5% CO_2	17·5 ± 2·0	3·9 ± 0·6	65·5 ± 11·4	Milsom & Jones (1980)
eudemys scripta	20	3–5% CO_2	6·8 ± 0·4	4·2 ± 1·3	28·3	Present study
studo horsfieldi	23–25	4% CO_2	15·7	5·3	82·6	Benchetrit & Dejours (1980)

* Calculated from reported values of f and $\dot{V}_T$.
Standard error shown when reported.

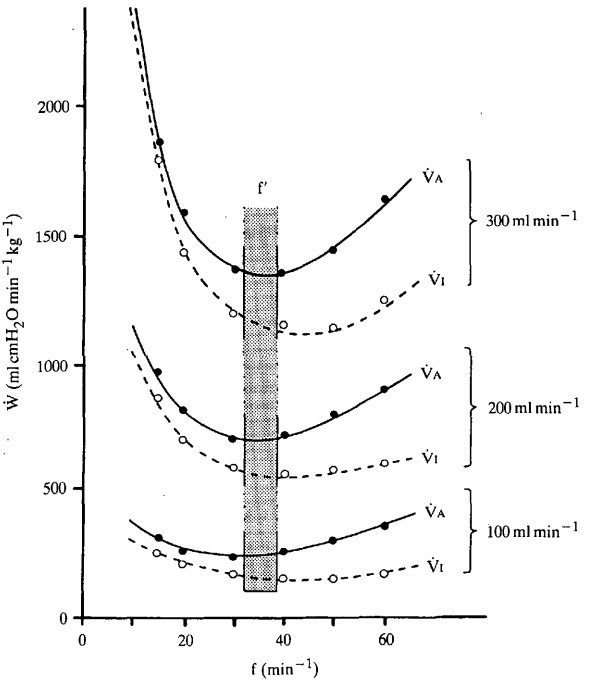

Fig. 3. The rate of work ($\dot{W}$) required to produce various constant levels of alveolar ventilation ($\dot{V}_A$ in ml min^{-1}) and total ventilation ($\dot{V}_I$ in ml min^{-1}) as a function of breathing frequency. The curves are derived from data presented in Vitalis & Milsom (1986) and the shaded area represents the range of instantaneous breathing frequencies ($f' = 60/T_{tot}$) measured in spontaneously breathing animals in the present study.

(Vitalis & Milsom, 1986). The power required to maintain a constant level of alveolar ventilation is plotted, along with that required to maintain a constant level of $\dot{V}_I$, as a function of pump frequency (f) in Fig. 3. Note that, due to dead space ventilation, $\dot{W}$ is always greater for any given level of $\dot{V}_A$ when compared to a similar level of $\dot{V}_I$, and that this difference increases as f increases because of rising non-elastic forces. The shaded area in Fig. 3 indicates the range of f′ calculated for spontaneously breathing animals. It can be seen that the f′ of spontaneously breathing animals corresponds closely with the pump frequencies which require the minimum rate of work to maintain a constant level of $\dot{V}_A$. They correspond less well to those frequencies at which the minimum work is required to maintain a constant level of total ventilation ($\dot{V}_I$). This observation suggests that during the episodes of continuous breathing seen in these turtles, spontaneous breathing patterns correspond closely to predicted patterns based on mechanical considerations.

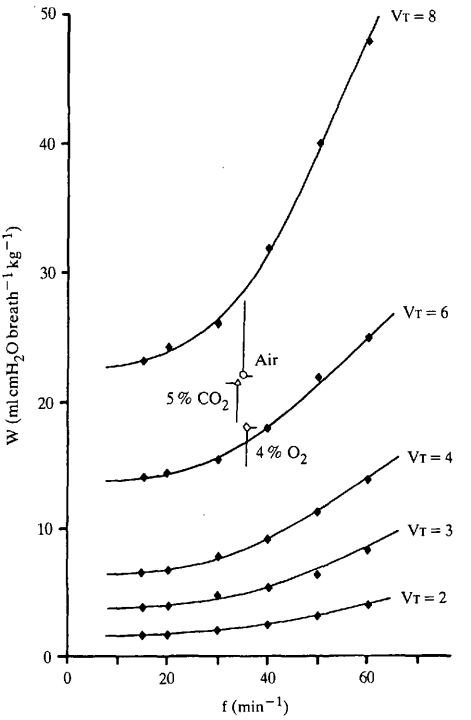

Fig. 4. The relationship between the work per breath (W) and pump ventilation frequency (f in cycles min^{-1}) for various levels of VT. The curves are derived from data presented in Vitalis & Milsom (1986). The open symbols represent the mean values ± S.E.M. of VT and instantaneous breathing frequency (f′) measured from six animals spontaneously breathing air (○), 5% CO_2 in air (△) or 4% O_2 in N_2 (◇) in the present study.

Marine turtles and tortoises do not breathe in bursts, as seen in *P. scripta*, but rather take single breaths interspersed with periods of breath holding (Shelton *et al.* 1985). Assuming the pulmonary mechanics and respiratory variables of these species are similar to those of *P. scripta*, the work of breathing associated with such a pattern can also be determined.

Fig. 4 shows the mechanical cost required to produce single breaths at various levels of VT as a function of instantaneous frequency ($60/T_{tot}$). The values of VT and f′ for intact *P. scripta* spontaneously breathing various gas mixtures are placed on the graph for comparison. These work-per-breath curves, derived from data in Vitalis & Milsom (1986), illustrate that for any given frequency, the smaller the VT, the lower the mechanical work of each breath. As pointed out earlier, however, a low VT

compromises alveolar ventilation and gas exchange. The need to keep VT sufficiently large in order to maintain alveolar ventilation, on the one hand, and the increased mechanical work associated with increases in VT, on the other, undoubtedly interact to produce the resting level of VT.

At any given VT, the work per breath is a function of breathing frequency. For a constant VT, the mechanical cost of breathing is reduced as the breathing frequency is lowered. There are physiological and behavioural factors, however, which would restrict breath duration from becoming too long. A very long breath in a spontaneously breathing animal would require a slow controlled inspiration and expiration. Not only would the stored elastic energy which partially powers both inspiration and expiration be lost, but both inspiratory and expiratory muscles would remain active in both phases of the ventilation cycle. Although the actual mechanical work of such a spontaneous breath would not be very different from the work recorded when expiration is passive, the oxidative cost would be much greater. This would result in a drop in the efficiency of ventilation. As many of the muscles which power ventilation in Chelonia are principally involved in locomotion, with an accessory role in ventilation, a prolonged breath duration would require that they spend a greater proportion of time on this accessory role (Gaunt & Gans, 1969). Another important consequence would be the loss of the non-ventilatory period, which would greatly restrict dive lengths in the aquatic and semi-aquatic species. All of these factors probably play important roles in restricting breath duration. Inspection of Fig. 4 shows, however, that given the shape of the relationship between work per breath and f, the decrease in mechanical work gained when frequency falls below the f′ measured in spontaneously breathing animals is very small compared with the large increase in the work per breath associated with an increase in breathing frequency above that value. This position of the f′ measured in spontaneously breathing animals on the work-per-breath curve may thus represent a compromise between mechanical and biological constraints.

It is clear that pulmonary vagal afferent information plays a key role in regulating the VT and f′ of each breath (Table 1, also see Milsom & Jones, 1980). Removal of lung stretch receptor information by vagotomy interrupts the negative feedback regulation of tidal volume and thus causes large increases in the mechanical work per breath. There is no evidence, however, that vagal afferent information plays a direct role in 'sensing' the level of mechanical work.

It has been suggested that the mechanism of the perception of the effort of breathing in humans lies in the muscle afferents of the respiratory system (Zechman & Wiley, 1966) which are involved in sensing the length–tension relationship in the respiratory muscles in response to imposed loads. Whether this also holds true for turtles is unclear. The most likely location for such sensors in turtles would be in the muscle afferents of the abdominal muscles and proprioceptors in the pelvic girdle. Whatever is the case, in spontaneously breathing turtles, if vagal information is removed, the work per breath increases sixfold due to an increase in VT and a drop in f′. This must result in an increased oxidative cost for ventilating the lungs. If ventilation is stimulated in a vagotomized animal through hypoxia or hypercapnia,

the work per breath increases a further two- and threefold, respectively, over values recorded in intact animals, due to further increases in VT and decreases in f'.

The data presented above appear to support the hypothesis that the periodic breathing patterns exhibited by reptiles represent an adaptive mechanism which minimizes the cost of breathing. In these animals continuous breathing is not required to meet metabolic demands, and the oxidative cost is high [10–30 % of total oxygen requirements in turtles (Kinney & White, 1977)]. When oxygen requirements rise or ventilation is stimulated by hypoxia or hypercapnia, it is less costly to restrict changes in VT and f' and take more breaths of similar length and depth (in a burst or individually), and thus shorten the non-ventilatory period, than it is to increase VT or shorten T_{tot}. The hypothesis would predict that the latter (shortening T_{tot}) will only occur when ventilation is so elevated that all periods of breath-holding disappear. This remains to be tested.

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REFERENCES

BENCHETRIT, B. & DEJOURS, P. (1980). Ventilatory CO_2 drive in the tortoise *Testudo horsfieldi*. *J. exp. Biol.* **87**, 229–236.

CROSFILL, M. L. & WIDDICOMBE, J. G. (1961). Physical characteristics of the chest and lungs and the work of breathing in different mammalian species. *J. Physiol., Lond.* **158**, 1–14.

GANS, C. & HUGHES, G. M. (1967). The mechanism of lung ventilation in the tortoise *Testudo graeca* Linne. *J. exp. Biol.* **47**, 1–20.

GAUNT, A. S. & GANS, C. (1969). Mechanics of respiration in the snapping turtle, *Chelydra serpentina* (Linne). *J. Morph.* **128**, 195–228.

GLASS, M. L., BOUTILIER, R. G. & HEISLER, N. (1983). Ventilatory control of arterial P_{O_2} in the turtle *Chrysemys picta bellii*: Effects of temperature and hypoxia. *J. comp. Physiol.* **151**, 145–153.

GLASS, M. L. & WOOD, S. C. (1983). Gas exchange and control of breathing in reptiles. *Physiol. Rev.* **63**, 232–260.

JACKSON, D. C. (1973). Ventilatory response to hypoxia in turtles at various temperatures. *Respir. Physiol.* **18**, 178–187.

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

KINNEY, J. L. & WHITE, F. N. (1977). Oxidative cost of ventilation in a turtle, *Pseudemys floridana*. *Respir. Physiol.* **31**, 327–332.

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

MILSOM, W. K. (1984). The interrelationship between pulmonary mechanics and the spontaneous breathing pattern in the Tokay lizard, *Gekko gecko*. *J. exp. Biol.* **113**, 187–201.

MILSOM, W. K. & JONES, D. R. (1980). The role of vagal afferent information and hypercapnia in control of the breathing pattern in Chelonia. *J. exp. Biol.* **87**, 53–63.

MILSOM, W. K. & VITALIS, T. Z. (1984). Pulmonary mechanics and the work of breathing in the lizard, *Gekko gecko*. *J. exp. Biol.* **113**, 187–202.

SHELTON, G. S., JONES, D. R. & MILSOM, W. K. (1985). Control of breathing in lower vertebrates. In *Handbook of Physiology*; *The Respiratory System*, vol. II, *Control of Breathing*, part 2 (ed. N. Cherniak & J. Widdicombe). Bethesda, Maryland: American Physiological Society.

VITALIS, T. Z. & W. K. MILSOM, (1986). Respiratory mechanics and the work of breathing in the semi-aquatic turtle, *Pseudemys scripta*. *J. exp. Biol.* **125**, 137–155.

ZECHMAN, F. W. & WILEY, R. L. (1968). Afferent inputs to breathing: respiratory sensation. In *Handbook of Physiology*; *The Respiratory System*, vol. II, *Control of Breathing*, part 1 (ed. N. Cherniak & J. Widdicombe). Bethesda, Maryland: American Physiological Society.