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THE EFFECT OF ALTERING PULMONARY BLOOD FLOW ON PULMONARY GAS EXCHANGE IN THE TURTLE *TRACHEMYS* (*PSEUDEMYS*) *SCRIPTA*

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Summary

In resting reptiles, the P_{O_2} of pulmonary venous return (P_{LAO_2}; left atrial blood) may be 20 mmHg (1 mmHg=0.1333 kPa) lower than the P_{O_2} of gas in the lung. This level of P_{O_2} is considerably higher than that observed in resting mammals and birds and results from ventilation–perfusion ($\dot{V}/\dot{Q}$) heterogeneity, pulmonary diffusion limitation and intrapulmonary shunting. However, the relative contribution of each of these factors is unknown. Many reptiles, particularly chelonians, exhibit an intermittent ventilation pattern where pulmonary blood flow ($\dot{Q}_L$) increases during the ventilatory periods and, therefore, we hypothesized that $\dot{V}/\dot{Q}$ matching would improve with increasing $\dot{Q}_L$. We applied the multiple inert gas elimination technique in anaesthetized turtles at 22 °C. Turtles were continuously ventilated at a rate of 140 ml kg^{-1} min^{-1}, equivalent to the rate of ventilation within a ventilatory period. Trace amounts of six inert gases were infused through the jugular vein. Blood samples from the pulmonary artery and the left atrium and mixed expired gases were collected for analysis. $\dot{Q}_L$ was reduced by a factor of six (low flow) using a vascular occluder placed around the common pulmonary artery or increased by a factor of two (high flow) through bolus injection of adrenaline. $\dot{V}/\dot{Q}$ heterogeneity was significantly reduced with increasing pulmonary blood flow (P<0.05). Consistent with these changes, the effective lung–pulmonary artery P_{O_2} difference (P_{LO_2}–P_{LAO_2}) was reduced (P<0.05) from 58±16 mmHg to 29±5 mmHg (means ± S.E.M.) and P_{LAO_2} increased significantly (P<0.05) from 88±17 mmHg (low flow) to 120±14 mmHg (high flow). There was evidence of pulmonary diffusion limitation under all conditions, which was unchanged with increasing blood flow. These findings suggest that increased pulmonary blood flow during a ventilatory period results in both temporal and spatial matching of ventilation and perfusion, without altering pulmonary diffusion limitation.

Key words: intrapulmonary shunts, reptiles, ventilation–perfusion heterogeneity, multiple inert gas elimination technique, turtle, *Trachemys scripta*.

Introduction

Many ectothermic vertebrates exhibit an intermittent breathing pattern characterized by long non-ventilatory periods interrupted by brief ventilatory periods consisting of one to several breaths (Shelton *et al.* 1986). During lung ventilation, pulmonary blood flow increases up to fivefold and thereby provides a temporal matching of lung perfusion and ventilation (Shelton and Boutilier, 1982; Shelton and Burggren, 1976; White and Ross, 1966). The increase in pulmonary blood flow during lung ventilation will affect both O_2 and CO_2 exchange (Burggren, 1987; Hicks and Wang, 1995); however, the physiological consequences of this cardio-respiratory coupling are unclear. One possibility is that the increase in pulmonary blood flow, *per se*, improves the spatial matching of ventilation and perfusion and thus improves the overall gas exchange efficiency of the lung (West *et al.* 1992; Wood, 1984). This possibility, although never tested experimentally, is appealing for reptiles, where the mean lung gas–end capillary blood P_{O_2} difference may be greater than 20 mmHg, a value that is considerably larger that the equivalent difference observed in resting birds and mammals (Burggren and Shelton, 1979; Glass, 1991; Hicks and White, 1992). The large P_{O_2} differences measured in reptiles could result from ventilation–perfusion ($\dot{V}/\dot{Q}$) heterogeneity, pulmonary diffusion limitation and intrapulmonary shunting, although the contribution of each of these factors is virtually unknown. The purpose of this study was to test the hypothesis that increases in pulmonary blood flow, *per se*, reduce $\dot{V}/\dot{Q}$ heterogeneity in the lung of the turtle *Trachemys* (*Pseudemys*) *scripta*. We used the multiple inert gas elimination technique to determine the spatial distribution of ventilation and perfusion and to quantify the degree of pulmonary diffusion limitation and intrapulmonary shunting at different pulmonary blood flows in the turtle.

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Materials and methods

This study was approved by the Animal Subjects Committees of the University of California, Irvine. Six turtles *Trachemys* (*Pseudemys*) *scripta* (Gray) (mass 1.95±0.18 kg, mean ± S.E.M.) were obtained from a commercial dealer, housed in large tanks in a temperature-regulated room (28 °C) and fed goldfish. On the morning of the experiment, anaesthesia was induced by injection with Nembutal (35 mg kg^{-1}, intramuscularly), and the animal was placed on its back and ventilated (SAR-830 ventilator, CWE Inc., Ardmore, PA) with 21 % O_2, 3 % CO_2 (balance N_2) at 140 ml kg^{-1} min^{-1}. The heart and central arteries were exposed by removing a 4 cm×4 cm piece of the plastron in order to implant flow probes and catheters. Ultrasonic flow probes (2R Transonic Systems Inc., Ithaca, NY) were placed around the left pulmonary artery and around the left aortic arch. Pulmonary flow was calculated by doubling the flow from the left pulmonary arch, and systemic flow was calculated by multiplying the flow from the left aortic arch by a factor of 2.8 (Comeau and Hicks, 1994; Shelton and Burggren, 1976). The common pulmonary artery and the left atrium were non-occlusively cannulated with PE 50 tubing (Ishimatsu *et al.* 1988) for direct sampling of blood flowing to and returning from the lung. The right jugular vein was occlusively cannulated for infusion of inert gases. A vascular occluder (model OC5A, In Vitro Metric, Healdsburg, CA) was placed around the common pulmonary artery. Ventilation was measured in triplicate at the beginning and end of the experiment by collecting expired gases in a Mylar gas-impermeable bag. Mixed expired and O_2 and CO_2 concentrations were also determined (Beckman OM-11, Sensormedics LB2, respectively).

Ventilation–perfusion distributions were obtained using the multiple inert gas elimination technique (Hlastala, 1984; Wagner *et al.* 1974*a*,*b*) modified for reptiles (Hopkins *et al.* 1995). A mixture of six inert gases (sulphur hexafluoride SF_6, ethane, cyclopropane, enflurane, diethyl ether and acetone) was dissolved in normal saline and infused *via* the jugular vein (rate 0.4–0.6 ml min^{-1}). Mixed expired gases were collected with Mylar gas-impermeable bags and transferred into gas-tight glass syringes. Duplicate 3 ml blood samples for the inert gas analysis were taken from the pulmonary artery and left atrium. Solubilities, retentions (*R* equal to the ratio of left atrial to mixed venous partial pressure) and excretions (*E* equal to the ratio of mixed expired to mixed venous partial pressure) for the inert gases were determined using gas chromatography (Hewlett-Packard 5890) (Wagner *et al.* 1974*a*,*b*), and $\dot{V}/\dot{Q}$ distributions were calculated from the inert gas data assuming an alveolar lung model. Using the multiple inert gas elimination technique, compartmental ventilation or blood flow to areas of different $\dot{V}/\dot{Q}$ ratios can be calculated and represented graphically. Conventionally, a logarithmic scale is used for these data, and the standard deviation of the perfusion distribution ($\log_{SD\dot{Q}}$) is used as an indicator of the degree of $\dot{V}/\dot{Q}$ heterogeneity (i.e. the greater the $\log_{SD\dot{Q}}$, the greater the $\dot{V}/\dot{Q}$ heterogeneity).

Left atrial ($P_{LA}O_2$) and pulmonary mixed venous ($P_{P\bar{V}}O_2$) P_{O_2}, P_{CO_2} and pH (Radiometer BMS3 MK2) were measured at 22 °C immediately after sampling. Correction factors for O_2 to allow for the difference between the electrode reading of the humidified calibration gas and the same gas tonometered in blood were determined by tonometry (DL213) of the blood of each animal on the day of the experiment.

Pulmonary blood flow was decreased slowly by inflating the pulmonary vascular occluder until flow was reduced to approximately one-sixth of the initial non-manipulated value. Pulmonary blood flow was increased by bolus injection of adrenaline (dose 0.1 μg kg^{-1}; Comeau and Hicks, 1994) into the left atrium. The animals were studied under anaesthesia, at 22 °C, during initial non-manipulated conditions, after 10 min of low pulmonary blood flow and after 5 min of increased pulmonary blood flow. The order of the low-flow and high-flow conditions was randomized.

Data were analyzed for differences between conditions using analysis of variance (ANOVA) for repeated measures using a commercially available software package (SuperANOVA 1.11, Abacus Concepts Inc., Berkeley, CA). Since pulmonary blood flow varied between animals under the control and high-flow conditions, additional regression analyses (StatView 4.1, Abacus Concepts Inc., Berkeley, CA) were also performed to examine the relationship between pulmonary blood flow, $P_{LA}O_2$ and the inert gas data. Seventeen observations from the six animals were used in the regression analyses. The effects of between-subject differences on the dependent and independent variables were controlled for by assigning dummy variables to encode the different subjects and performing a multiple regression (Glantz and Slinker, 1990). Significance was accepted at $P<0.05$ (two-tailed, unless specified one-tailed in text). Results are reported as means ± S.E.M.

Results

Complete data were obtained on six animals for control and high-flow conditions and are summarized in Tables 1 and 3. In one animal, we were not able to position the vascular occluder and therefore report data for five animals in the low-flow condition.

Blood flow, metabolic and ventilatory data

Pulmonary and systemic blood flows are given in Fig. 1. Initial (control) pulmonary blood flow varied markedly from animal to animal as shown by the high standard error. From control conditions, we were able to decrease pulmonary flow to one-sixth of the initial value and to increase it by a factor of almost two ($P<0.001$). Systemic blood flow did not change significantly throughout the experiment. Ventilation and metabolic data are given in Table 1. Ventilation was held constant throughout the experiment, and there were no significant changes in oxygen consumption rate ($\dot{V}_{O_2}$), CO_2 production rate ($\dot{V}_{CO_2}$) or respiratory exchange ratio during the study.

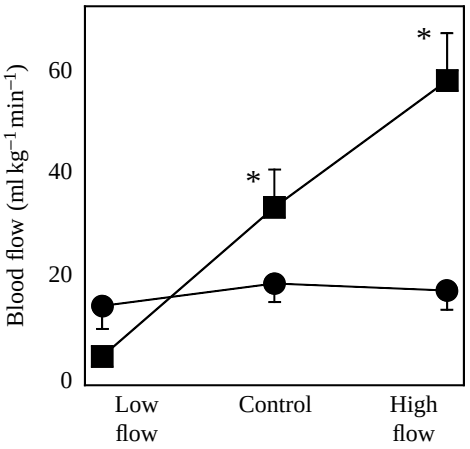

Fig. 1. Pulmonary and systemic blood flow across experimental conditions. Filled squares, pulmonary blood flow; filled circles, systemic blood flow. Values are means ± S.E.M., N=6; low flow, N=5. An asterisk marks values that are significantly different from the low-flow value for pulmonary blood flow; two-tailed t-test, P<0.05.

Table 1. *Ventilatory and metabolic data under conditions of reduced pulmonary blood flow (low flow), no manipulation of flow (control) and increased pulmonary blood flow (high flow)*

Condition	Low flow (N=5)	Control (N=6)	High flow (N=6)
$\dot{Q}_L$ (ml kg^{-1} min^{-1})	5.7±1.4	35.2±7.6	60.3±9.4**
$\dot{V}_E$ (ml kg^{-1} min^{-1})	146±28	142±23	142±23
$\dot{V}_{O_2}$ (ml kg^{-1} min^{-1})	0.54±0.12	0.52±0.11	0.55±0.13
$\dot{V}_{CO_2}$ (ml kg^{-1} min^{-1})	0.14±0.03	0.22±0.08	0.25±0.07
Effective lung P_{O_2} (mmHg)	147±1	148±2	149±1*
P_{LAO_2} (mmHg)	88±17	115±6	120±14*
Effective P_{LO_2}–P_{LAO_2} difference (mmHg)	58±16	33±6	29±5*
P_{LACO_2} (mmHg)	15.8±3.4	16.4±3.1	16.6±3.0
pH$_{LA}$	7.82±0.13	7.85±0.16	7.79±0.12
$P_{P\bar{V}O_2}$ (mmHg)	32±11	65±8	80±7**
$P_{P\bar{V}CO_2}$ (mmHg)	16.3±3.5	16.6±3.1	16.6±3.1
pH$_{P\bar{V}}$	7.79±0.12	7.80±0.11	7.78±0.11

Values are means ± S.E.M.
1 mmHg = 0.1333 kPa.
Significantly different across conditions: *P<0.05, **P<0.01.
$\dot{Q}_L$, pulmonary blood flow; $\dot{V}_E$, minute ventilation; $\dot{V}_{O_2}$, rate of oxygen consumption; $\dot{V}_{CO_2}$, rate of carbon dioxide production; P_{LAO_2}, partial pressure of oxygen in left atrial blood; P_{LACO_2}, partial pressure of carbon dioxide in left atrial blood; pH$_{LA}$, pH in left atrial blood; $P_{P\bar{V}O_2}$, partial pressure of oxygen in pulmonary mixed venous blood; $P_{P\bar{V}CO_2}$, partial pressure of carbon dioxide in pulmonary mixed venous blood; pH$_{P\bar{V}}$, pH in pulmonary mixed venous blood.

Blood gases

Blood gas data obtained from the pulmonary artery and left atrium are given in Table 1. There was a significant increase (one-tailed, P<0.05) in P_{LAO_2} with increasing pulmonary blood flow, associated with a significant increase in $P_{P\bar{V}O_2}$ (P<0.001). There were no other significant changes in blood gas levels. P_{LAO_2} was significantly correlated with log(pulmonary blood flow) (Fig. 2, r=0.68, P<0.05) and changes in P_{LAO_2} were reflected in $P_{P\bar{V}O_2}$ (r=0.72, P<0.0001). Effective lung P_{O_2} was calculated from the mixed expired gas concentrations and the inert gas dead space (Table 1) and increased significantly (P<0.05) with increasing pulmonary blood flow, although the changes were small. The difference between calculated effective P_{LO_2} and P_{LAO_2} was significantly reduced with increasing pulmonary blood flow (one-tailed, P<0.05), and there was a significant negative correlation between log(pulmonary blood flow) and the effective P_{LO_2}–P_{LAO_2} difference (r=–0.67, P<0.05).

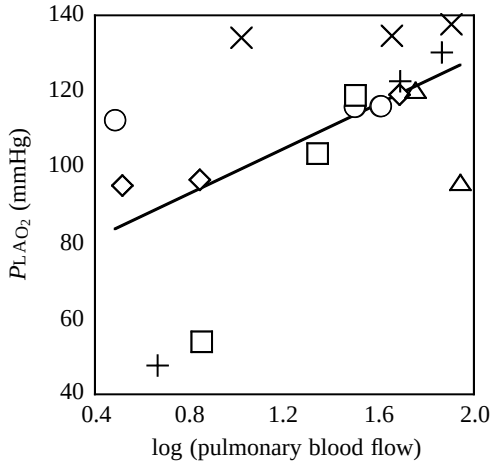

Fig. 2. Linear regression of P_{LAO_2} (in mmHg; 1 mmHg=0.1333 kPa) *versus* log(pulmonary blood flow) (in ml kg^{-1} min^{-1}). Different symbols indicate data from separate animals. r=0.68, P<0.05, y=69.1+29.7x.

Table 2. *Blood gas partition coefficients measured at 22.4±0.32 ˚C*

Gas	Partition coefficient
SF_6	0.00620±0.00025
Ethane	0.0832±0.0022
Cyclopropane	0.579±0.012
Enflurane	1.95±0.04
Diethyl ether	25.2±0.9
Acetone	498±33

Hematocrit = 18.3±1.55.
*N = 6; Values are means ± S.E.M.

Ventilation–perfusion relationships

Blood gas partition coefficients for the six inert gases at 22 °C are presented in Table 2. The lower solubilities for SF_6, ethane and cyclopropane and the higher solubilities for the remaining gases, compared with mammalian values, are probably the result of the low haematocrits in these animals (18.1±1.6) (Young and Wagner, 1979). Inert gas data are

Table 3. *Inert gas data under conditions of reduced pulmonary blood flow (low flow), no manipulation of flow (control) and increased pulmonary blood flow (high flow)*

Condition	Low flow (N=5)	Control (N=6)	High flow (N=6)
RSS	2.0±0.9	3.1±0.3	7.5±4.1
$\dot{Q}_{shunt}$ (% of $\dot{Q}_L$)	11.0±3.2	16.7±2.0	19.5±3.7
$\dot{Q}_{low\ \dot{V}/\dot{Q}}$ (% of $\dot{Q}_L$)	1.1±0.7	7.5±6.1	12.4±12.4
$\dot{Q}_{shunt} + \dot{Q}_{low\ \dot{V}/\dot{Q}}$ (% of $\dot{Q}_L$)	12.1±2.9	24.2±7.6	32.0±11.7
Mean of $\dot{Q}$ distribution	5.88±2.23	1.42±0.37	0.70±0.16*
$\log SD_{\dot{Q}}$	0.95±0.18	0.79±0.19	0.54±0.08*
Mean of $\dot{V}$ distribution	9.27±3.33	2.31±0.56	1.57±0.55*
$\log SD_{\dot{V}}$	0.63±0.12	0.67±0.21	0.74±0.26
V_D (% of ventilation)	45.4±4.9	27.1±4.3	23.9±2.4**
P_{LAO_2} (p-o) (mmHg)	21±9	16±3	16±5

Values are means ± S.E.M.

Significantly different across conditions: *P<0.05, **P<0.01.

RSS, residual sum of squares; $\dot{Q}_{shunt}$, blood flow to areas of intrapulmonary shunt; $\dot{Q}_{low\ \dot{V}/\dot{Q}}$, blood flow to areas of low $\dot{V}/\dot{Q}$ (ratio <0.1); $\log SD_{\dot{Q}}$, log standard deviation of the perfusion distribution; $\log SD_{\dot{V}}$, log standard deviation of the ventilation distribution; V_D, dead space ventilation; P_{LAO_2} (p-o), partial pressure of oxygen in left atrial blood predicted from the inert gases minus the measured P_{LAO_2}.

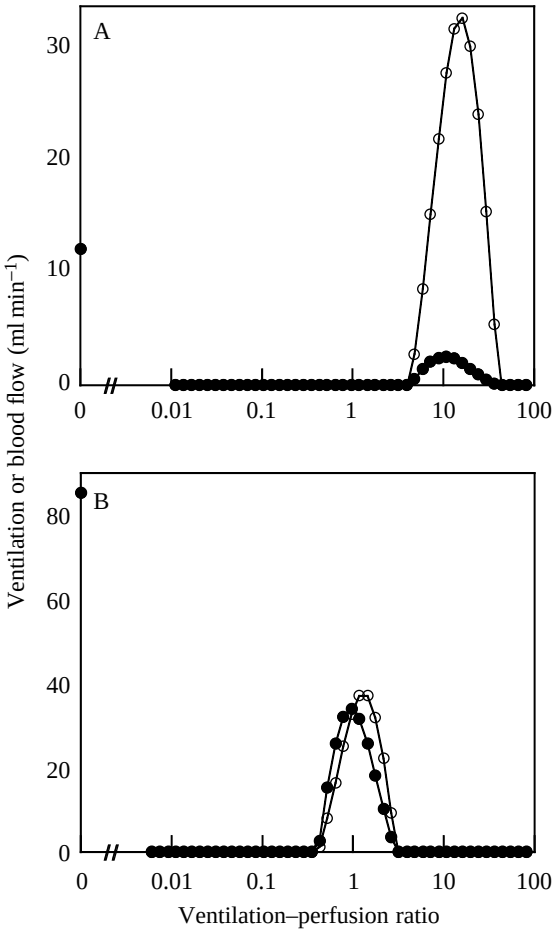

Fig. 3. (A,B) Recovered $\dot{V}/\dot{Q}$ distribution from one representative animal under (A) low-flow conditions, where log standard deviation of the perfusion distribution ($\log SD_{\dot{Q}}$) is 1.07, indicating moderate to marked $\dot{V}/\dot{Q}$ heterogeneity, and (B) high-flow conditions, where $\log SD_{\dot{Q}}$ is 0.48, indicating little $\dot{V}/\dot{Q}$ heterogeneity. Open circles are ventilation in (ml min^{-1}), filled circles are perfusion (in ml min^{-1}) to each of 50 compartments of different $\dot{V}/\dot{Q}$ ratio, ranging from 0 (shunt) to infinity (dead space).

summarized in Table 3. The residual sum of squares (RSS) is a measure of the goodness of fit of the inert gas data and is also an indicator of the technical adequacy of the data. The RSS follows a χ^2 distribution and, with random experimental error, should be ⩽16.8 in 99 % of occurrences with six gases, although the RSS can also be increased in the presence of incomplete intrapulmonary gas mixing. However, there was no evidence for this, as all RSS data were within acceptable limits (Powell and Wagner, 1982). Fig. 3 shows a typical measured $\dot{V}/\dot{Q}$ distribution during both low and high pulmonary blood flow conditions. The mean of the perfusion distribution decreased significantly (P<0.05) with increasing pulmonary blood flow. There was a significant decrease in $\log SD_{\dot{Q}}$ with increasing blood flow (one-tailed, P<0.05) and there was a significant relationship between $\log SD_{\dot{Q}}$ and log(pulmonary blood flow) (Fig. 4, r=–0.69, P<0.05), suggesting that $\dot{V}/\dot{Q}$ matching is improved when pulmonary blood flow is increased.

Perfusion to areas of the lung with a low $\dot{V}/\dot{Q}$ ratio ($\dot{V}/\dot{Q}$ ratio less than 0.1) was 1 % of pulmonary blood flow during the low pulmonary flow condition and averaged 12 % during the high pulmonary flow condition. The difference between experimental conditions was not significant (Table 3). Intrapulmonary shunting varied from 11 % under the low pulmonary flow conditions to almost 20 % during the high pulmonary flow condition, but these differences were not significant (Table 3). Note that the catheter placement in the pulmonary artery and the left atrium excludes any contribution from cardiac shunts. Inert gas dead space decreased significantly with increasing pulmonary blood flow (P<0.01) (Table 3, V_D). Inert gas dead space includes anatomical dead space and instrument dead space, unlike physiological dead space, which also includes ventilation to some areas of high $\dot{V}/\dot{Q}$.

Left atrial P_{O_2} was predicted for each animal on the basis of the observed $\dot{V}/\dot{Q}$ distribution and intrapulmonary shunt, and assuming diffusion equilibrium (Table 3) (Torre-Bueno, 1985). We used published data (Maginniss *et al.* 1980) on the oxygen dissociation curve of the turtle to modify the inert gas subroutine to allow for the difference in shape between the turtle and human oxygen dissociation curves. The turtle, unlike most mammals, has multiple haemoglobins and, therefore, the following data are subject to the limits of the approximation of the oxygen dissociation curve by the inert gas subroutine and must be interpreted with some caution. The P_{O_2} predicted from the derived $\dot{V}/\dot{Q}$ distribution was significantly greater than the

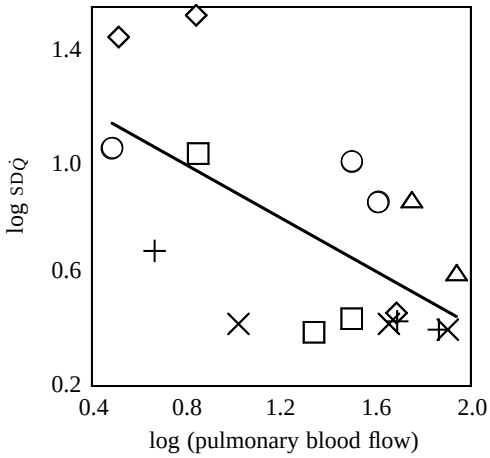

Fig. 4. Linear regression of ventilation–perfusion heterogeneity ($\log SD_{\dot{Q}}$) *versus* log(pulmonary blood flow) (in ml kg^{-1} min^{-1}). Different symbols indicate data from separate animals. r=–0.69, P<0.05, y=1.41–0.49x.

observed $P_{LA O_2}$ at all levels of pulmonary blood flow (P<0.001), suggesting pulmonary diffusion limitation. The predicted P_{O_2} did not change across conditions and was not significantly correlated with pulmonary blood flow.

Discussion

In anaesthetized pump-ventilated turtles, we found that increasing pulmonary blood flow resulted in a decrease in ventilation–perfusion heterogeneity and an increase in left atrial P_{O_2}. This is the first study to investigate the effect of pulmonary blood flow on $\dot{V}/\dot{Q}$ relationships in an animal that markedly varies its blood flow under resting conditions.

Cardio-respiratory synchrony in turtles

Many reptiles, particularly turtles, are intermittent lung breathers. In these animals, brief ventilatory periods are interspersed among apnoeas of variable duration (Shelton *et al.* 1986). During apnoea, a bradycardia develops and there is a significant increase in the pulmonary vascular resistance, which leads to a reduction in $\dot{Q}_L$. For example, in the freely diving turtle *Trachemys* (*Pseudemys*) *scripta*, $\dot{Q}_L$ may be as low as 10–15 ml kg^{-1} min^{-1} (Shelton and Burggren, 1976). In contrast, during brief ventilatory periods, the cardiovascular changes are the reciprocals of those occurring during apnoea. In *Trachemys* (*Pseudemys*) *scripta*, the heart rate increases, the pulmonary vascular resistance decreases and $\dot{Q}_L$ can be as large as 50–70 ml kg^{-1} min^{-1} (Heisler *et al.* 1983; Shelton and Burggren, 1976). In the present study, we were able to alter pulmonary blood flow over a wide range (from 6 to 60 ml kg^{-1} min^{-1}) and to measure the direct effects of these changes on pulmonary gas exchange.

Adrenaline has many effects and it is possible that the injection of the drug in the present study might have effects on the pulmonary vascular bed other than vasodilation. Adrenaline was chosen to increase pulmonary blood flow for the following reasons. In the rat snake *Elaphe obseleta*, recent studies have shown that electrical stimulation of vagal nerves produces a pulmonary vasoconstriction, followed by a pulmonary vasodilation during the post-stimulatory period (Donald *et al.* 1990). The post-stimulatory vasodilation is unaffected by atropine, but is eliminated following administration of propranolol (Donald *et al.* 1990). In turtles, stimulation of vagal afferents results in a twofold increase in pulmonary blood flow (Comeau and Hicks, 1994), which is attenuated following infusion of bretylium. These results suggest that the increase in pulmonary blood flow associated with intermittent lung ventilation may result from adrenergic vasodilation (Comeau and Hicks, 1994; Donald *et al.* 1990; Lillywhite and Donald, 1989).

The reduction in pulmonary blood flow during apnoea is under cholinergic control (Burggren, 1985; Comeau and Hicks, 1994; Hicks, 1994; White, 1976). In chelonians, approximately 50 % of the variable vascular resistance lies proximal to the lung parenchyma (Burggren, 1985). This has been demonstrated by *in situ* perfusion of distal pulmonary arteries, which show a strong cholinergic vasoconstriction and adrenergic vasodilation (Burggren, 1985). An additional important site for the regulation of pulmonary resistance is the bulbus cordis, a ‘sphincter-like’ internal wrapping of smooth muscle and an external covering of cardiac muscle at the base of the pulmonary artery (Burggren, 1977; March, 1961). The underlying smooth muscle is constricted by cholinergic agonists and dilated by adrenergic agonists (Burggren, 1985).

Pulmonary blood flow and ventilation–perfusion heterogeneity

In the simplest approach, a single-compartment model, the mean alveolar ventilation divided by the mean blood flow can be used to determine the $\dot{V}/\dot{Q}$ ratio. Although this approach has been used in previous studies in reptiles (Wood *et al.* 1977), it is of limited value, since it assumes that the lung is perfectly homogeneous with respect to $\dot{V}/\dot{Q}$ and neglects the effect of the distribution of $\dot{V}/\dot{Q}$ ratios within the many thousands of subunits within a lung. For example, a lung could have an overall $\dot{V}/\dot{Q}$ ratio centred at 1; however, if there were marked heterogeneity about this mean ratio, pulmonary gas exchange would be substantially impaired. Conversely, if the mean $\dot{V}/\dot{Q}$ ratio were low, yet blood flow was well matched to the available ventilation, with little heterogeneity, the overall effect on gas exchange would be minimized. The effect of increasing $\dot{V}/\dot{Q}$ heterogeneity is to decrease both pulmonary oxygen uptake and carbon dioxide excretion, resulting in a decrease in P_{O_2} and an increase in P_{CO_2} in the blood returning to the left atrium. Thus, most modern research focuses on the spatial distributions of $\dot{V}/\dot{Q}$ ratios rather than the overall $\dot{V}/\dot{Q}$ ratio of the lung, and several review articles explore this subject (Hlastala and Robertson, 1978; West, 1969; West and Wagner, 1991). Using the multiple inert gas technique, the distribution of ventilation and perfusion to areas of differing $\dot{V}/\dot{Q}$ ratio can be represented graphically, and the dispersion or heterogeneity of blood flow and ventilation about the mean is

given by the standard deviation of the distribution on a logarithmic scale ($\log SD_{\dot{Q}}$ or $\log SD_{\dot{V}}$, respectively).

In normal mammalian lungs, $\log SD_{\dot{Q}}$ is almost always less than 0.5 (Gale *et al.* 1985); it may reach as high as approximately 0.7 during exercise in normal athletic humans (Hopkins *et al.* 1994) and approach 2.0 in patients with chronic obstructive pulmonary disease (West and Wagner, 1991). Ventilation–perfusion heterogeneity has been measured in a number of reptile species with values for $\log SD_{\dot{Q}}$ ranging from less than 0.5 in the alligator (Powell and Gray, 1989) and monitor lizard (Hopkins *et al.* 1995) to over 0.9 in the tegu lizard (Hlastala *et al.* 1985). In the turtle under control and low-flow conditions, there was moderate to marked $\dot{V}/\dot{Q}$ heterogeneity; however, this improved with increasing pulmonary flow to values approaching that of the alligator and monitor lizard.

The reason for a worsening of $\dot{V}/\dot{Q}$ matching under conditions of low flow is uncertain. In the mammalian lung, inter-regional $\dot{V}/\dot{Q}$ heterogeneity may occur, where changes in the pulmonary vascular–alveolar pressure relationships may result in increased $\dot{V}/\dot{Q}$ heterogeneity by increasing the area of the lung where the alveolar pressure exceeds the pulmonary arterial pressure (zone 1). In the turtle, however, different lung zones are unlikely, since gravity-dependent regional differences in blood flow are small and lung air space pressures are expected to be equal throughout. A second possibility is intra-regional $\dot{V}/\dot{Q}$ heterogeneity, where local changes in vascular impedance secondary to changes in pulmonary vascular recruitment may alter $\dot{V}/\dot{Q}$ relationships. In perfused dog lung preparations, where care was taken to keep the lung under zone 2 conditions (zone 2 conditions occur when pulmonary arterial pressure exceeds alveolar/lung air space pressure, and is expected in the turtle lung under the conditions of the present study) (Domino *et al.* 1991; Ohlsson *et al.* 1989), decreasing blood flow produced marked $\dot{V}/\dot{Q}$ heterogeneity ($\log SD_{\dot{Q}}$=1.00), suggesting that the effect of lowering pulmonary blood flow on $\dot{V}/\dot{Q}$ heterogeneity can occur in the absence of inter-regional changes in $\dot{V}/\dot{Q}$ heterogeneity. However, in the dog lung, in contrast to the turtle lung, increasing pulmonary flow resulted in increased $\dot{V}/\dot{Q}$ heterogeneity (Domino *et al.* 1991), possibly as a result of increased perfusion of corner vessels, increased perfusion to areas of high ventilation–perfusion ratio, or subtle pulmonary oedema resulting from the experimental procedure. Similar increases in $\dot{V}/\dot{Q}$ heterogeneity are seen in humans (Hammond *et al.* 1986) and varanid lizards (Hopkins *et al.* 1995) during exercise, where pulmonary blood flow and vascular pressures are increased, and may be the result of interstitial pulmonary oedema. The reason for the differences between mammals and turtles is unknown. However, turtles are one of the few vertebrates that have large changes in pulmonary blood flow associated with increasing ventilation, in the absence of exercise.

Intrapulmonary shunts and dead space ventilation

We measured an intrapulmonary shunt of 17 % under control conditions, which is similar to the value obtained by Seymour (1983) using 100 % oxygen breathing at a lung volume providing neutral buoyancy. Since the oxygen method for detecting shunt measures the contribution of desaturated blood to the output of the left heart, both the intrapulmonary shunt and the shunt from extrapulmonary sources will be measured. These extrapulmonary sources of shunting, such as the bronchial circulation, will extract oxygen from the blood and will therefore be measured as contributing to the oxygen shunt. However, since inert gases will not be extracted by the bronchial circulation, they will not measure these extrapulmonary contributors to the oxygen shunt. Therefore, the shunt measured by the oxygen method will be greater than the inert gas shunt, particularly at low levels of shunting where extrapulmonary shunting will contribute a greater proportion of the total shunt (Hlastala *et al.* 1975). In our experimental preparation, care was taken to exclude any contribution from cardiac shunts by the placement of the sampling catheters in the pulmonary artery and the left atrium, and we cannot comment on other sources of shunting since 100 % O_2 was not given. Unlike the varanid lizard, where increasing pulmonary blood flow with exercise resulted in a decreased intrapulmonary shunt (Hopkins *et al.* 1995), the intrapulmonary shunt in the present study tended to increase with increasing pulmonary blood flow. This may represent a difference between species or may result from the differing experimental means (exercise *versus* adrenaline) of increasing pulmonary blood flow.

Inert gas dead space includes anatomical dead space and instrument dead space. Dead space ventilation was significantly reduced when pulmonary blood flow was increased. This reduction is probably a result both of the increasing perfusion of areas of the lung with high $\dot{V}/\dot{Q}$ ratio with increasing pulmonary blood flow and of the recruitment of unperfused areas of the pulmonary circulation with increasing pulmonary blood flow.

Pulmonary diffusion limitation

The inert gas method allows an estimate of pulmonary diffusion limitation by computing the arterial and expired P_{O_2} that would be expected from the recovered $\dot{V}/\dot{Q}$ distribution. For each of the 50 compartmental $\dot{V}/\dot{Q}$ values, compartmental oxygen concentrations are calculated for the inspired and mixed venous gas concentrations. Assuming lung gas–end capillary diffusion equilibrium, the appropriately weighted mixed arterial and mixed expired concentrations of O_2 are calculated and can be compared with the measured values. When the observed value is less than that predicted from the inert gas exchange, this suggests pulmonary diffusion limitation. Another possible explanation for the discrepancy between observed and predicted O_2 concentration is extrapulmonary shunting (e.g. cardiac, bronchial arteries and thebesian veins), since this would also result in a lower P_{O_2} than that predicted from the $\dot{V}/\dot{Q}$ distribution. Note that the placement of the catheters in the pulmonary artery and left atrium would preclude any cardiac shunts, and the effect of

other sources of shunting is likely to be very small (Hlastala *et al.* 1975).

There was evidence of pulmonary diffusion limitation in the turtle, since the P_{O_2} of left atrial blood was 16–21 mmHg lower than that predicted from the recovered $\dot{V}/\dot{Q}$ distribution. Caution must be used in interpreting these data since the analyses are limited by our ability to approximate the oxygen dissociation curve of the turtle. We did not measure the oxygen dissociation curve in our animals, and therefore any deviation of these animals from published data (Maginniss *et al.* 1980) would be expected to affect the results. For example, if the P_{50} of the animal were overestimated, it would increase the predicted P_{LAO_2} and reduce the effective P_{LO_2}–predicted P_{LAO_2} difference; conversely, the effect of overestimating P_{50} would be to reduce the predicted P_{LAO_2}. These uncertainties in P_{50} are minimized in lungs with little $\dot{V}/\dot{Q}$ inequality and increased in lungs with marked inequality. In humans (Hammond *et al.* 1986; Hopkins *et al.* 1994) and the varanid lizard (Hopkins *et al.* 1995), pulmonary diffusion limitation is seen only during very heavy exercise. Although the degree of diffusion limitation was constant across conditions, since the degree of $\dot{V}/\dot{Q}$ heterogeneity and therefore the effective P_{LO_2}–P_{LAO_2} difference was reduced with increasing blood flow, the relative contribution of pulmonary diffusion limitation to the effective P_{LO_2}–P_{LAO_2} difference was larger at higher blood flow in the turtles.

Incomplete intrapulmonary gas mixing

In awake, spontaneously breathing varanid lizards (Hopkins *et al.* 1995), we found evidence of incomplete intrapulmonary gas mixing, and this has also been reported in the anaesthetized alligator (Powell and Gray, 1989). In the present study, our turtles were anaesthetized and ventilated at a high rate corresponding to the level of ventilation observed during a ventilatory episode. We did not find evidence of incomplete intrapulmonary gas mixing, as all residual sums of squares (RSS) were within the expected χ^2 distribution for six gases (Powell and Wagner, 1982). Incomplete intrapulmonary gas mixing will increase the RSS since the retention of the heaviest gas (enflurane) will be increased in comparison with that of gases of low molecular mass (cyclopropane) (Downs and Wagner, 1983). It is likely that the high ventilation rate that was used in these animals improved convective gas mixing within the lung and prevented incomplete intrapulmonary gas mixing. Also, the turtle may have additional mechanisms which prevent limitations of gas exchange, resulting from incomplete gas mixing during periods of apnoea. Spragg *et al.* (1980) found that movement of limbs and contraction of smooth muscle within the lung increased the displacement of gas from the posterior to the anterior parts of the lung and facilitated gas mixing.

Arterial blood gases

Increased pulmonary blood flow resulted in a decrease in $\dot{V}/\dot{Q}$ heterogeneity, a decrease in inert gas dead space and an unchanged intrapulmonary shunt and pulmonary diffusion limitation. The net effect was a reduction in the effective P_{LO_2}–P_{LAO_2} difference, an increase in P_{LAO_2} and improved pulmonary gas exchange. Although the intrapulmonary shunt was numerically greater in the high-flow than the low-flow conditions, the consequences of intrapulmonary shunting are minimized, since pulmonary mixed venous P_{O_2} was significantly increased from 32 mmHg under the low pulmonary flow conditions to 80 mmHg under high pulmonary flow conditions. Thus, the shunted blood is at a higher P_{O_2} and the effect on arterial P_{O_2} of combining shunted blood with mixed venous blood is reduced. Arterial P_{O_2} was also improved with increasing pulmonary blood flow because of reduced dead space ventilation and improved $\dot{V}/\dot{Q}$ matching. The net effect of the improvement in $\dot{V}/\dot{Q}$ heterogeneity was an increase in the predicted arterial P_{O_2} from 127 mmHg to 135 mmHg.

We can conclude that, in the anaesthetized turtle, intrapulmonary shunting, pulmonary diffusion limitation and $\dot{V}/\dot{Q}$ heterogeneity all contribute to the P_{O_2} difference between lung gas and left atrial blood. This is in contrast to the situation in mammals and varanid lizards, which do not show appreciable pulmonary diffusion limitation at rest. In the turtle, $\dot{V}/\dot{Q}$ heterogeneity is significantly reduced with increasing pulmonary blood flow and it appears that the increase in pulmonary blood flow observed with increasing ventilation in the awake animal probably results in reduced $\dot{V}/\dot{Q}$ heterogeneity and improved pulmonary gas exchange.

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