Arterial O_2 Homeostasis during Diving in the Turtle *Chelodina longicollis*

Warren Burggren[1]
Allan Smits[2]
Barbara Evans[3]
[1]Department of Zoology, University of Massachusetts, Amherst, Massachusetts 01003-0027; [2]Department of Biology, Box 19498, University of Texas at Arlington, Arlington, Texas 76019; [3]Department of Zoology, University of Melbourne, Parkville, Victoria 3052, Australia

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Abstract

Periodically breathing animals that store O_2 primarily in the lungs must efficiently transfer O_2 to arterial blood during apnea. Previous experiments on diving freshwater turtles indicate that such transferral is continuous during most dives, but there have been occasional observations of much more periodic O_2 transfer between lung gas and blood. This study investigates the dynamics of lung O_2 utilization and blood transfer during voluntary diving in the Australian long-necked turtle, Chelodina longicollis. *Pulmonary arterial blood flow was measured by a relatively noninvasive impedance technique. The P_{O_2} of pulmonary gas and systemic arterial blood was measured continuously with extracorporeal catheter loops, which minimize sampling disturbances. Lung gas P_{O_2} declined relatively constantly during apnea at a rate of about 3 mmHg/min. Changes in arterial blood P_{O_2} showed one of two very distinctive patterns during apnea. In the first pattern, evident in about two-thirds of 87 dives monitored in seven turtles, the P_{O_2} of arterial blood decreased from levels at the start of the dive at a rate of about 1.0–1.5 mmHg/min. In a second pattern observed in the remaining one-third of the dives, arterial P_{O_2} actually showed periods of transient increase of at least 4 mmHg at some point during the dive, while about 8% showed a transient P_{O_2} increase of 10 mmHg or more. This second pattern of arterial blood oxygenation was also quite distinctive in that arterial O_2 saturation was maintained constant at around 85%–95%, even when dives lasted for 20 or more min. Qualitative measurements of pulmonary blood flow during voluntary dives indicate that the transient increases in arterial blood P_{O_2} are closely correlated with large and equally transient increases in pulmonary perfusion. We suggest that* C. longicollis *can maintain constant arterial O_2 saturation during long periods of diving by periodically increasing pulmonary blood flow to transfer O_2 stored in lung gas into blood perfusing the lungs. Furthermore, we suggest that this may be a general phenomenon among diving reptiles but that its observation requires animals unstressed by sampling techniques.*

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Introduction

Vertebrates can store significant amounts of O_2 for use during diving. O_2 can be stored chemically bound to respiratory pigments for transport (hemoglobin) and storage (myoglobin) and can be stored in the form of gas remaining in the lungs at the start of the dive. Although there are exceptions, diving birds and mammals tend to store O_2 primarily in blood and tissues, with lung gas contributing relatively little to the total O_2 stores (Elsner and Gooden 1983; Burggren 1988). In amphibians and reptiles, however, as a result of the combination of mass-specific lung volumes that are larger than in birds and mammals (Burggren 1989; Perry 1989) and generally lower blood O_2 capacity, the largest proportion of O_2 at the start of diving resides within lung gas rather than within blood or tissue fluids (Burggren 1988).

Depending heavily on the lungs as an O_2 store during diving interjects an additional complication to O_2 transport during apnea. In birds and mammals, the major O_2-storage site and the transport medium are one and the same—the blood. Thus, the transfer of O_2 from the storage site to metabolizing cells will be achieved simply by perfusing arterial blood through the tissues. However, in amphibians and reptiles, O_2 must first be transferred from storage in lung gas to hemoglobin in pulmonary capillary blood before the majority of available O_2 can be transported to the systemic tissues.

The patterns and processes of O_2 transfer from lung gas to pulmonary blood in ectothermic vertebrates during diving have received considerable attention (for reviews, see Randall et al. 1980; Shelton and Boutilier 1982; Shelton 1985; Boutilier and Shelton 1986; Burggren 1988). The rate of O_2 transfer from lung gas to pulmonary capillary blood, manifested in the rate of fall of pulmonary gas P_{O_2}, is usually greatest in the first minutes of apnea, progressively declining as apnea continues. Presumably, this results from the facts that (1) the P_{O_2} gradient from lung gas to pulmonary arterial blood is largest at the beginning of apnea, decreasing as apnea progresses (Burggren and Shelton 1979), and (2) pulmonary perfusion generally decreases as apnea proceeds (Shelton 1976; White 1976; Burggren 1985, 1987). Changes in either or both of these factors during the dive could alter the rate of utilization of lung O_2 stores. Currently, it is not clear to what extent the rate of O_2 transfer from lung gas to arterial blood can be regulated once diving begins, although a few studies of gas transport in amphibians and reptiles have indicated that transfer of O_2 from lung gas to arterial blood during diving can be a discontinuous process, which suggests a regulatory capability. In one such study on voluntarily diving turtles, *Pseudemys scripta* (Burggren and Shelton 1979), simultaneous measurements of lung gas and arterial blood P_{O_2} occasionally revealed very distinctive deviations from the typical pattern of O_2 transfer from lung gas to arterial blood. During long

dives, in particular, periods of sudden, rapid decline in lung P_{O_2} and an actual sharp rise in arterial P_{O_2} punctuated periods of slower gradual decline in the P_{O_2} of both lung gas and arterial blood. The interpretation of these data has been that pulmonary perfusion transiently increased during apnea (though pulmonary blood flow was not measured), producing a brief enhancement of O_2 transfer from lung gas to pulmonary capillary blood (Burggren and Shelton 1979; White 1985; Burggren 1988). Occasional brief increases in pulmonary blood flow during diving have been observed by others in turtles (R. Silver and D. Jackson, unpublished) and snakes (Lillywhite and Donald 1987), though in those experiments lung or blood gases were not monitored. There is also indirect evidence for discontinuous transfer of O_2 from lung to blood stores during diving for the frog *Xenopus laevis*. Abrupt changes in the rate of decline of pulmonary P_{O_2} during apnea are suggestive of transient changes in the rate of pulmonary perfusion and/or P_{O_2} of pulmonary arterial blood (Boutilier and Shelton 1986; unpublished data of R. Boutilier, P. Butler, and B. Evans described in Burggren 1988).

Thus, while the process of transfer of O_2 from lung gas to arterial blood in lower vertebrates during diving generally has been observed to occur in a more or less steady, slow, and continuous fashion, there are anecdotal observations that indicate that utilization of lung O_2 stores may also occur in a more complex, periodic fashion. The objective of the present study was thus to test the hypothesis that diving turtles could exert control over the rate of O_2 transferral from lung gas to systemic arterial blood. We have modified techniques for comparatively nondisruptive measurements of gas and blood flow and have applied these techniques to the study of pulmonary gas exchange during diving in the Australian freshwater turtle *Chelodina longicollis*.

Material and Methods

Experiments were performed on 11 Australian long-necked turtles (*Chelodina longicollis*) collected in northern Victoria, Australia. The animals, which had a mean (±1 SD) body mass of 950 ± 254 g, were housed for 1–3 wk in shallow water at approximately 25°C and were fed chopped heart and liver.

Surgical Preparation

In preparation for cannula implantation, each turtle was first cooled in crushed ice. *Chelodina* rarely encounters environmental temperatures below 15°C, and being cooled to near 0°C rendered the animal completely

flaccid and unresponsive to mechanical stimuli. Lidocaine was then injected under the skin on the dorsal surface of the right rear leg. A 3-cm incision was made to expose the sciatic artery and vein, which were then occlusively cannulated with 60-cm lengths of PE 90 cannulae. Extensive collateral circulation exists in the leg of this turtle, and occlusive cannulation appeared to have little effect on normal limb function. Each cannula was filled with heparinized saline, and approximately 200 IU of heparin/kg body mass was injected into the circulation. The catheters were led out of the incision, which was closed with interrupted sutures.

A 1-cm-diameter hole was drilled through each side of the carapace over the lungs. Both lungs were cannulated dorsolaterally with 60-cm lengths of PE 200 cannulae, according to the technique of Burggren, Glass, and Johansen (1977), to allow withdrawal and return of lung gas samples. A copper wire impedance electrode (40 gauge, 0.5 mm diameter) was passed through each hole in the carapace and introduced into the pleural cavity just under the carapace to allow recording of pulmonary blood flow (see below). The holes in the carapace through which the cannulae and electrode wires emerged were then sealed with epoxy glue.

In three turtles, a 4-cm square hole was sawed in the plastron to allow implantation of an electromagnetic blood flow transducer (Shelton and Burggren 1976). The left pulmonary artery was exposed, and a 2.0- or 3.0-mm-diameter transducer (Zepeda; Seattle, Wash.) was fitted around it. The leads from the transducer were led out of the opening, and the excised piece of plastron was glued back in place with epoxy.

All of the leads and cannulae were led over the carapace and formed into a single "cable" to prevent turtles from becoming entangled in them. After all surgery was completed, animals were allowed to warm to room temperature (20°–22°C).

Measurement of Lung and Blood Gases and Blood Flow

Each turtle was placed in a 40-L aquarium filled with constantly aerated water at 20°–22°C (fig. 1). The top of the aquarium was covered with a floating Styrofoam lid which was perforated with a breathing hole 10 cm in diameter. The breathing hole was covered with an inverted funnel, the neck of which contained an airflow transducer used to monitor gas flow produced by inspiration and expiration. A constant flow of air was maintained through the funnel at a level sufficient to prevent the turtle from rebreathing any exhaled gas. The leads and cannulae from the turtle emerged from the aquarium via a separate small hole in the lid. Thus, each turtle was unrestrained and free to dive and surface at will. The aquarium was kept in constant subdued light and shielded from movements of the investigators. Each turtle was given at

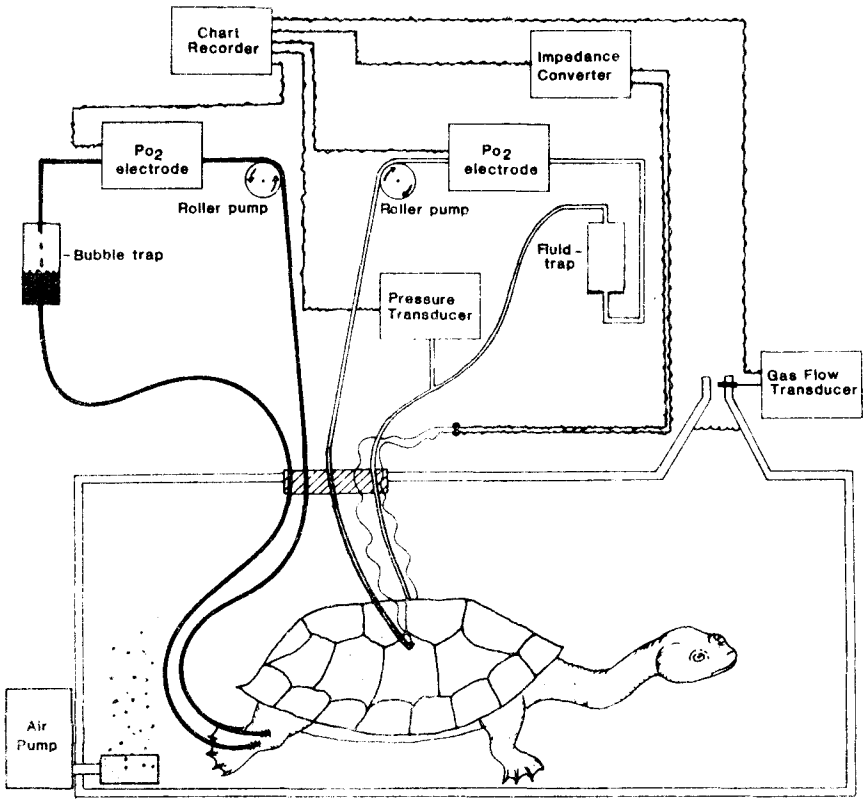

Fig. 1. Experimental apparatus used in these experiments. The Po_2 of arterial blood and of pulmonary gas was monitored continuously by using roller pumps to pass blood and gas through cuvettes containing O_2 electrodes. An impedance convertor was used to monitor pulmonary blood flow (in three turtles, electromagnetic flow transducers—not shown—were also used to measure pulmonary blood flow). Changes in intrapulmonary pressure were monitored with a pressure transducer attached to a side arm of the pulmonary catheter, while lung ventilation was monitored with a gas flow transducer placed across the mouth of a breathing hole in the aquarium. See text for further details.

least 12 h (and often 16–20 h) to recover from surgery in the aquarium before the leads and cannulae were connected and measurement begun. In every case, this recovery period resulted in a heart and lung ventilation rate similar to that observed in resting turtles not subjected to surgery.

The copper wire electrodes from the left and right pleural cavity were connected to a Biocom impedance converter. The leads from the electromagnetic blood flow transducers (when present) were connected to a Zepeda SWF-4 Flowmeter. The output from both of these devices was delivered to preamplifiers of a Grass Model 7 polygraph.

In order to monitor continuously arterial P_{O_2}, the sciatic arterial cannula was connected to one channel of a Watson-Marlow multichannel peristaltic pump set to draw arterial blood at a rate of 0.69 mL/min. Blood from the pump entered a cuvette containing a Radiometer P_{O_2} electrode and went on to a gas-bubble trap before entering the cannula in the sciatic vein for return to the turtle. Hematocrit was measured (see below) before arterial blood P_{O_2} was monitored and at the end of the experiments in six turtles to assess whether the extracorporeal loop was producing erythrocyte hemolysis. Hematocrit at the end of the experiments was usually at least 90% of its initial value, and the colorless centrifuged plasma indicated no significant erythrocyte hemolysis.

In order to monitor continuously lung gas P_{O_2}, one of the pulmonary cannulae was connected to one channel of the peristaltic pump, which withdrew gas (0.40 mL/min) from one lung and passed it on to a second Radiometer P_{O_2} cuvette. Gas from the cuvette entered a small fluid trap and was then returned to the other lung via the other lung cannula. The volume of the entire pulmonary gas loop was less than 1% of lung volume in every experiment. The outflow arm of a T-piece placed in the pulmonary cannula was connected to a Validyne differential pressure transducer, whose output was amplified and displayed on the chart recorder. This pressure signal, which reflected ambient hydrostatic pressure on the body surface during diving and the rapid excursions in intrapulmonary pressure during lung ventilation, gave an independent corroboration of the occurrence and timing of lung ventilation events monitored by the airflow transducer located in the funnel over the breathing hole.

Both Radiometer P_{O_2} electrodes were connected to Radiometer PHM72 electrometers, whose outputs were displayed either on the Grass chart recorder or a two-channel SP-G3C Speedex flatbed recorder. In vitro experiments involving rapid movement of the catheter tips between gases and blood of various P_{O_2}'s were used to establish transit times through the catheters to the O_2 electrodes. The transit time for the standard length of arterial cannula was 35 s. Transit time in the pulmonary gas cannulae was 50 s. Lag times due to cannula transit were nullified in all of the figures shown in this article by horizontally shifting the blood gas and lung gas recordings leftward by the appropriate number of seconds relative to the recordings of lung pressure and blood flow. No corrections were made to account for O_2 electrode response time, but 95% response time for both P_{O_2} measurement systems was less than 50 s.

Correlation of Techniques for Measuring Blood Flow

A qualitatively accurate index of pulmonary blood flow was deemed at the outset to be critical to interpreting changes in lung and blood gas. Yet mini-

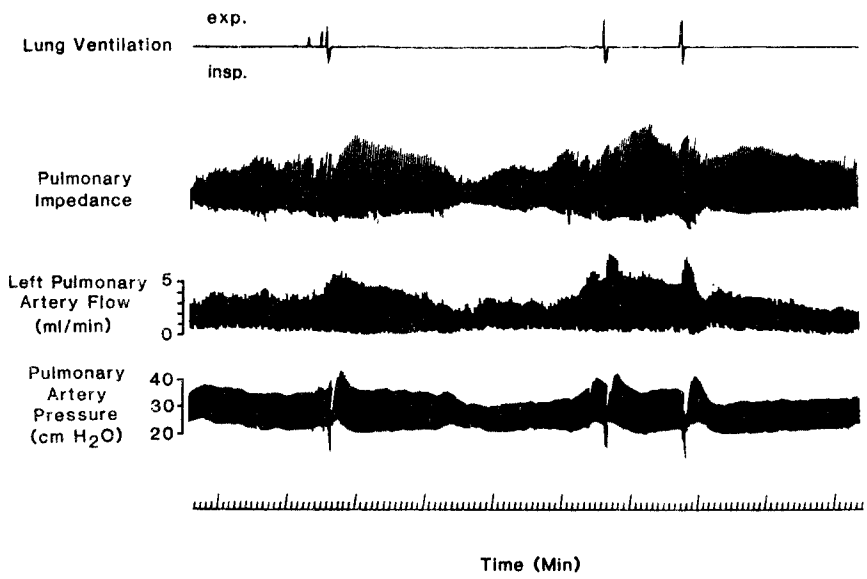

Fig. 2. Records of lung ventilation, left pulmonary artery flow and common pulmonary artery pressure measured in an unrestrained, voluntarily breathing and diving turtle, Chelodina longicollis *(body mass 1,500 g). Also indicated is the pulmonary impedance signal monitored via two copper wire electrodes placed in the upper regions of the left and right pleural cavities. Although beat-to-beat differences exist, the pulmonary impedance signal closely reflects changes in pulmonary blood flow measured with an electromagnetic transducer. See Material and Methods for further information.*

mally invasive techniques were desirable. Thus, impedance techniques for monitoring arterial blood flow were modified for use in diving turtles. Impedance signals from the copper wire electrodes introduced into the left and right pleural cavity showed very sharp, distinct oscillations that appeared in exact phase with heartbeat, as determined by ECG and/or measurement of arterial blood pressure. To establish the extent of correlation between the magnitude of the impedance signal and magnitude of blood flow, experiments were performed on three turtles in which each animal was instrumented with both pulmonary impedance electrodes and an electromagnetic flow probe located on the left pulmonary artery. As evident in figure 2, there was a consistent and close correlation of the pulmonary impedance signal and blood flow measured by the electromagnetic flow probe over a wide range of flows produced by voluntary intermittent lung ventilation and apnea. While beat-to-beat differences between the two types of blood flow signals certainly occurred, the average height of the impedance signal over successive 10-s periods followed mean arterial flow very closely

in 19 dives monitored in each of the three animals fitted with both methods for monitoring blood flow. Consequently, the less invasive technique of measuring pulmonary impedance was assumed to provide a *qualitative* indication of changes in pulmonary blood flow during ventilation and apnea. No attempt was made to calibrate the impedance signal or to compare impedance signals between animals.

Blood Respiratory Properties

The hematology and respiratory properties of the blood were determined in order to facilitate interpretation of the blood gas data. After all in vivo monitoring of gas partial pressures and blood flow was completed, approximately 3 mL of blood was withdrawn from each animal. Hematocrit was determined on a 10-μl. sample by centrifugation at 10,000 rpm. The remaining blood was separated into two samples, which were then equilibrated with either 21% O_2/3% CO_2 or 0% O_2/3% CO_2. O_2 equilibrium curves were constructed with a "mixing" technique, in which P_{O_2} was measured in blood samples containing known fractions of 100% O_2-saturated and 0% O_2-saturated blood. This allowed % O_2 saturation of blood to be plotted against P_{O_2}. Blood P_{O_2} was measured with a Radiometer P_{O_2} electrode and pHM72 electrometer. The pH at 50% saturation (P_{50}) was measured with a Radiometer pH electrode. After constructing an O_2 equilibrium curve with blood equilibrated with 3% CO_2, we reequilibrated the blood with <0.05% CO_2, producing a higher pH at P_{50}. A second O_2 equilibrium curve was constructed and the Bohr effect calculated. Finally, we reconstituted the O_2 equilibrium curve of that animal's blood at pH 7.70, using each animal's Bohr effect and Hill equation.

Results

Blood Respiratory Properties

Oxygen equilibrium curves were constructed from blood drawn from six *Chelodina longicollis* (body mass 530 ± 314 g). Figure 3 summarizes these data, which reveal hematological and respiratory characteristics that are comparable with those of other freshwater turtles (Burggren, Hahn, and Foex 1977).

Changes in Lung P_{O_2} during Diving

Simultaneous, continuous measurements of the P_{O_2} of lung gas and systemic arterial blood during undisturbed, voluntary diving and breathing were ob-

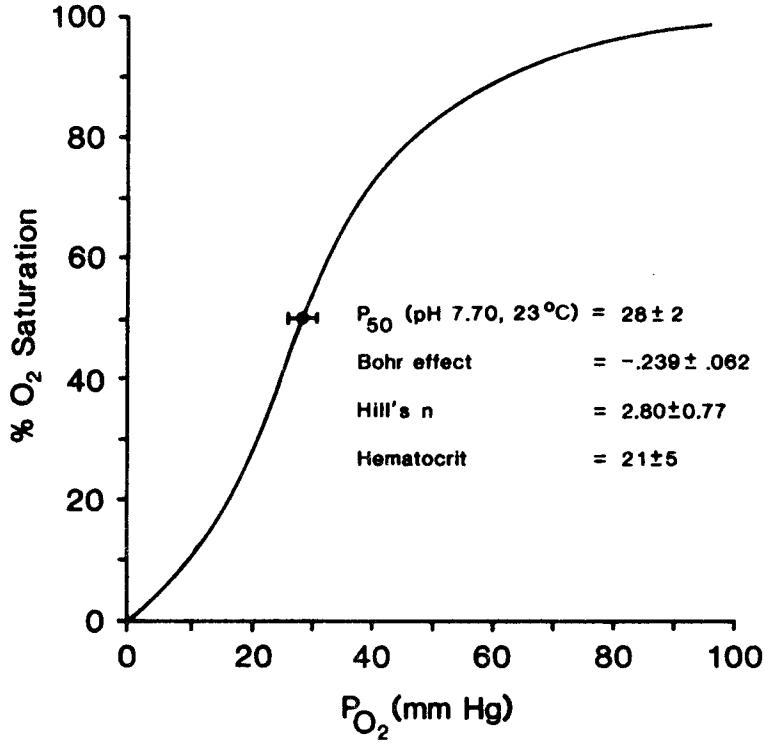

Fig. 3. The O_2 equilibrium curve of Chelodina longicollis, *measured at 23° C and pH 7.7. The mean values (± 1 SD) of P_{50}, Bohr effect, Hill's n, and hematocrit calculated from blood drawn from six turtles are also indicated.*

tained from 87 dives by seven turtles. Dive lengths were highly variable, ranging from less than 1 min to as long as 1 h. About 50% of all measured dives were longer than 5 min.

Lung gas P_{O_2} changed in a highly predictable fashion with intermittent breathing, not only from dive to dive in an individual but also when the seven turtles were compared. Figure 4 shows representative traces of the P_{O_2} of lung gas and arterial blood during six consecutive dives in a 948-g *C. longicollis*. The lung P_{O_2} in this and all other animals was highest within the first minute following the termination of lung ventilation and the start of diving, and then began a slow, consistent fall at a rate of approximately 3 mmHg/min. There was very little deviation from this rate of decline at any point during the dive, and the sudden sharp decreases or plateaus in lung gas P_{O_2} reported by Burggren and Shelton (1979) in *Pseudemys scripta* were never observed in *C. longicollis*.

Changes in Systemic Arterial P_{O_2} during Diving

Arterial P_{O_2} at the start of diving was about 50–70 mmHg below that of lung gas, a finding consistent with previous studies on freshwater turtles and in-

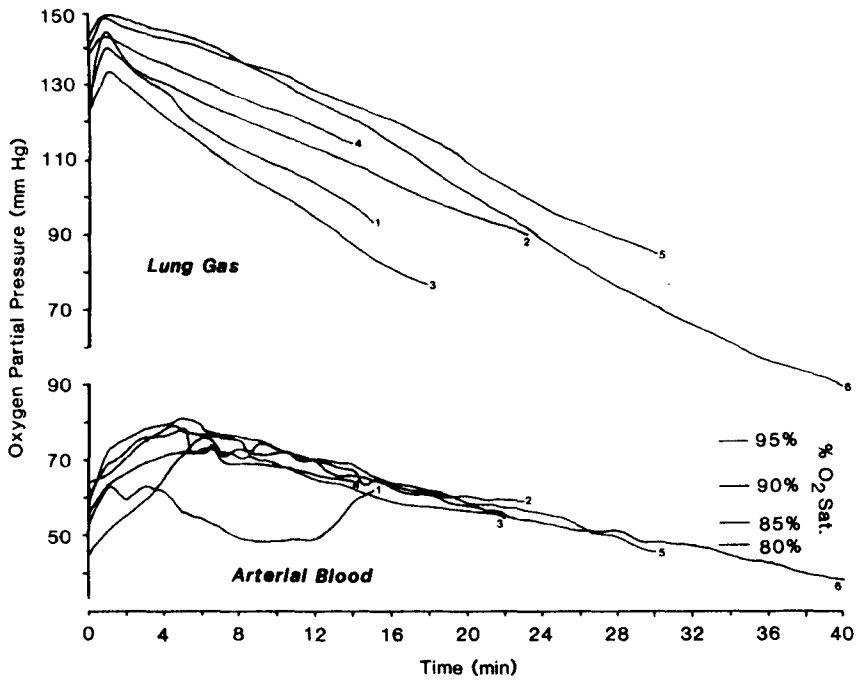

Fig. 4. Changes in the P_{O_2} of lung gas and systemic arterial blood beginning at the onset of a brief period of lung ventilation and the ensuing dive in a 948-g Chelodina longicollis. *In every case the dive began before 2 min on the time scale. The six pairs of tracings represent data from six successive dives. The lower right-hand portion of the diagram contains O_2 saturation isopleths determined from the in vitro O_2 equilibration curve in fig. 2. No correction for changes during the dive resulting from the Bohr effect has been made.*

dicative of both physiological shunts within the lungs and right-to-left intracardiac shunts (Burggren and Shelton 1979). Changes in P_{O_2} of systemic arterial blood during diving were much less consistent than changes in lung gas P_{O_2}. In essence, two different patterns emerged.

The first pattern is characterized by dives 2–6 in figure 4. Arterial P_{O_2} was still increasing as diving began and, unlike lung gas P_{O_2}, did not reach maximum values until 2–4 min after lung ventilation stopped and diving began. This 1–3-min delay between peak lung gas P_{O_2} and peak arterial blood P_{O_2} may reflect, in part, the circulation time of blood between the lungs and the peripheral sampling point for systemic arterial blood. The major characteristic of dives of this first pattern was that arterial P_{O_2} decreased consistently during the dive at a rate of about 1.0–1.5 mmHg/min. Small transient changes in systemic arterial P_{O_2} were sometimes imposed on the overall

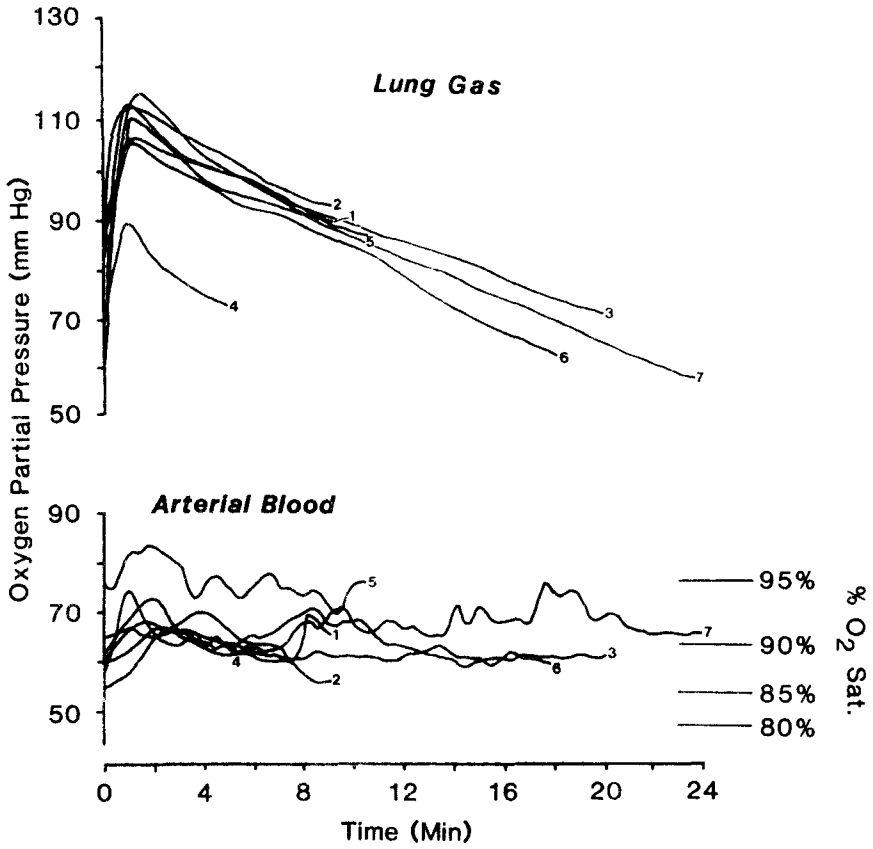

Fig. 5. Changes in the P_{O_2} of lung gas and systemic arterial blood beginning at the onset of a brief period of lung ventilation and the ensuing dive in a 595-*g* Chelodina longicollis. *Seven consecutive diving cycles are shown. Data are presented as described in the legend to fig. 4.*

decline in P_{O_2} during the dive but were generally less than 1–2 mmHg in magnitude.

To facilitate interpretation of these measured P_{O_2} changes, the O_2 equilibrium curve depicted in figure 3 was used to determine isopleths for blood O_2 saturation. These O_2 isopleths (which do not account for any Bohr shift that might occur during the course of the dive) are located in the right portion of figure 4. During a dive showing the first pattern of arterial O_2 depletion, blood O_2 saturation typically fell from a high of about 95% at the start of diving to about 80% after 30 min and to about 35%–40% after 50 min.

The second, quite different pattern of arterial P_{O_2} change during diving is evident in dive 1 in figure 4 and during the seven consecutive dives by a 595-g turtle that are depicted in figure 5. In this pattern of diving, arterial P_{O_2} was far more labile, showing numerous transient upward and downward

excursions. In dive 1 in figure 4, P_{O_2} at first decreased during the dive but then showed a period of 4–5 min with no change before actually starting to rise again to a P_{O_2} and % O_2 saturation that were equivalent to those in the first minutes of the dive. In the seven dives illustrated in figure 5, although systemic arterial P_{O_2} showed numerous rapid changes upward and downward, the % O_2 saturation of the blood showed little change during the course of diving, remaining between 85% and 95% even after 20 min of apnea. We have thus referred to this second diving pattern as representing arterial O_2 homeostasis. Observation of the turtles during diving (with care taken that the researchers not be observed in turn) indicated that the turtles showed little locomotor activity during diving. The occasional sporadic movements of the limbs or head could never be correlated with transient changes in either pulmonary blood flow or systemic arterial oxygenation.

In order to quantify the incidence of occurrence of these two different diving patterns, all 87 monitored dives by seven turtles were categorized according to whether increases in systemic arterial P_{O_2} occurred after the initial postdive peak. Dives were classified according to whether they showed less than a 4-mmHg transient increase in arterial P_{O_2} during the dive, showed an incremental increase of 4–9 mmHg, or showed an incremental increase of more than 10 mmHg. Sixty-four percent of all dives showed either incremental increases of less than 4 mmHg in systemic arterial P_{O_2} during the dive or a progressive decrease in P_{O_2} during the dive. Representing the second class of arterial O_2 homeostasis, 37% of all dives showed at least one incremental increase of 4 mmHg or more in P_{O_2}, and, of these dives, 8% showed an increase of 10 mmHg or more. Two of seven turtles exhibited dives in which the systemic arterial P_{O_2} soared more than 20 mmHg within a few seconds at some point during a voluntary, undisturbed dive.

Blood Flow and Arterial P_{O_2} during Diving

Chelodina longicollis proved to be like other freshwater turtles that have been examined, with periods of lung ventilation accompanied by a tachycardia of variable magnitude and a large increase in pulmonary perfusion (figs. 2, 6, and 7). When the diving pattern typified by progressive arterial depletion (fig. 4) occurred, pulmonary blood flow decreased slowly and smoothly in the minutes following the initiation of diving. Pulmonary blood flow showed few transient adjustments during the dive, and those that occurred were of very small magnitude.

Quite different patterns of pulmonary blood flow were measured in dives reflecting the second diving pattern (arterial O_2 homeostasis), in which arterial blood P_{O_2} remained relatively fixed at a high % O_2 saturation throughout

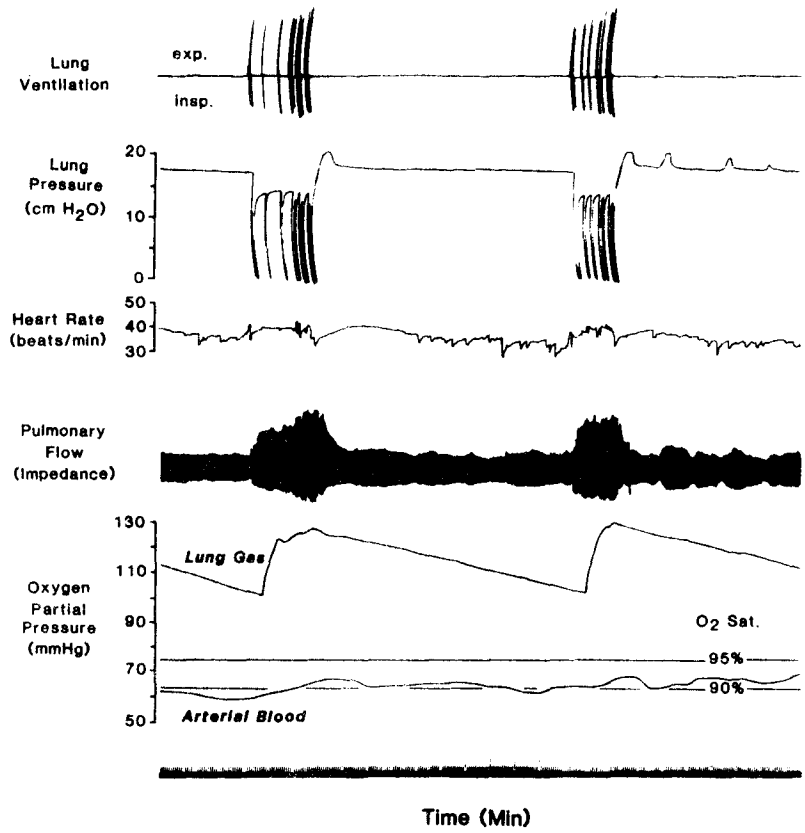

Fig. 6. Records of lung ventilation (monitored by gas flow at a breathing hole), intrapulmonary lung pressure, heart rate (triggered from the pulmonary impedance signal), pulmonary flow (monitored by pulmonary impedance electrodes), and the P_{O_2} of lung gas and systemic arterial blood in an unrestrained, undisturbed Chelodina longicollis *(body mass 835 g). O_2 isopleths are indicated in the lower right-hand portion of the diagram.*

the dive. In this dive pattern, showing maintained arterial P_{O_2}, blood flow to the lungs was elevated during lung ventilation but then fell sharply on the initiation of diving. Typically, small, transient increases in blood flow lasting less than 1 min would occur throughout apnea (e.g., after the second bout of lung ventilation in fig. 6), while at the same time arterial P_{O_2} and % O_2 saturation oscillated around a broadly constant level.

Graphic evidence of the close relationship between pulmonary blood flow and systemic arterial P_{O_2} during diving is presented in figure 7. In the left-hand panel, two closely spaced bursts of air breathing are accompanied by the typically brief rise in pulmonary blood flow. Lung gas P_{O_2} began to increase almost immediately, and, after a delay related primarily to the circulation time between the pulmonary veins and the peripheral systemic circu-

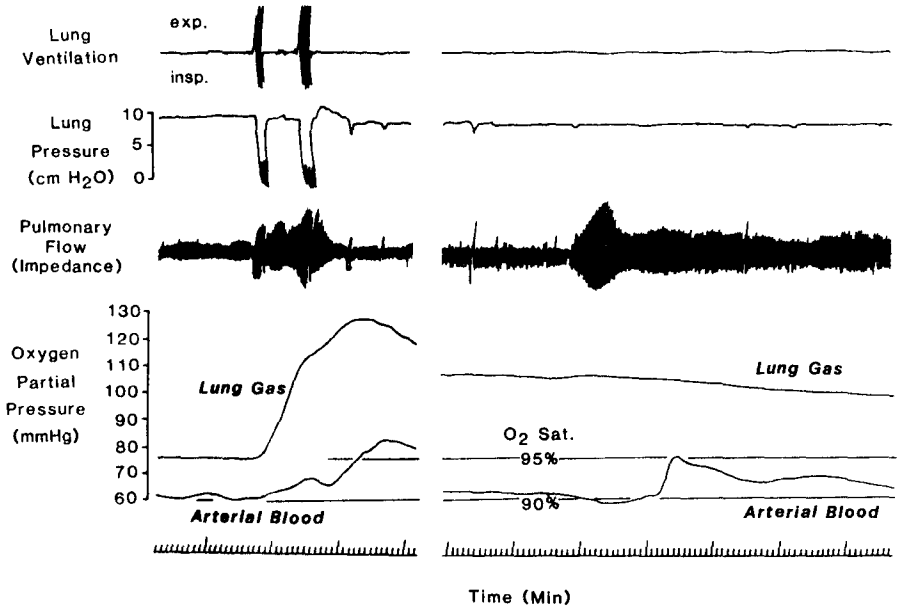

Fig. 7. Records of lung ventilation, intrapulmonary lung pressure, pulmonary flow, and the P_{O_2} of lung gas and systemic arterial blood in an unrestrained, undisturbed Chelodina longicollis *(body mass 595 g). The left-hand panel shows records at the termination of a dive and during two brief periods of lung ventilation. The right-hand panel shows records taken between 9 and 16 min of diving. O_2 saturation isopleths are indicated in the lower region of the right-hand panel.*

lation, systemic arterial P_{O_2} also began to increase. In this particular dive, blood flow fell very sharply as a long dive commenced. The right-hand panel shows the continued low pulmonary blood flow approximately 10 min into this dive. In this particular dive, however, pulmonary blood flow spontaneously began to increase at 10 min, rising rapidly to levels that, on the basis of the impedance signal, were at or above the levels seen before the dive began. After about a 1-min delay (once again reflecting primarily the blood circulation time), P_{O_2} increased nearly 20 mmHg within a 15–20-s period. An elevated pulmonary blood flow continued for several minutes, and during this time arterial P_{O_2} hovered between 70 and 75 mmHg (90%–95% O_2 saturation). During long dives exhibiting arterial O_2 saturation, several such pulsatile increases could develop before the next series of lung ventilations began.

Discussion

Chelodina longicollis shows two quite distinctive patterns of O_2 transfer and utilization during diving. One pattern, which accompanied the majority of

dives and was typical of previous observations on diving reptiles, is characterized by a continual slow depletion of O_2 from both lung gas and systemic arterial blood. However, in the second, less common pattern, *Chelodina* has demonstrated a striking ability to maintain the O_2 saturation of systemic arterial blood at levels approaching full saturation throughout dives lasting as long as ½ h, a pattern we have termed arterial O_2 homeostasis. Aerobic metabolism of the tissues must be continuing throughout these dives, because the O_2 store in lung gas is being continually depleted, as evidenced by the continuous fall in the P_{O_2} of lung gas. Our interpretation is that O_2 is being transferred from lung gas to systemic arterial blood at the same overall rate as O_2 is being transferred from systemic arterial blood to the metabolically active tissues. Systemic arterial oxygenation is thus in a steady state.

How are these two distinct patterns of oxygen utilization produced? What are their possible physiological advantages and disadvantages?

The first question, dealing with the mechanism(s) producing these patterns of arterial oxygenation, is the more straightforward. Although gas exchange in the lungs of at least one species of freshwater turtles has a measurable diffusion limitation (Burggren and Shelton 1979), gas exchange in vertebrate lungs is primarily perfusion-limited. Consequently, an increase in pulmonary blood flow during diving will increase the rate of O_2 transfer from lung gas to pulmonary capillary blood, enhancing the oxygenation of (or decreasing the rate of O_2 depletion from) systemic arterial blood. The common observation in *C. longicollis* of a close correlation of a spontaneous and abrupt increase in pulmonary blood flow with a sharp, transient rise in systemic arterial blood flow shortly thereafter (fig. 7) indicates that the rate of pulmonary blood flow is an important factor influencing systemic arterial O_2 saturation. The observation in turtles (R. Silver and D. Jackson, unpublished) and snakes (H. Lillywhite, unpublished) of occasional brief increases in pulmonary blood flow during diving indicates that the potential exists for other reptiles to "meter out" O_2 from the lung gas store by adjusting pulmonary perfusion during diving.

An increase in pulmonary blood flow preceding a rise in systemic arterial O_2 saturation occurred so routinely in *Chelodina* as to establish unequivocally their causal interrelationship. Yet it is important to note that increased pulmonary blood flow did not universally result in an increase in systemic arterial P_{O_2}, nor were changes in arterial blood P_{O_2} always preceded by a surge in pulmonary blood flow. This suggests that other factors could contribute to arterial O_2 homeostasis. While this study does not report on intracardiac shunting or central venous and arterial O_2 saturations, these also are critical elements affecting pulmonary gas exchange. In the absence of any change in pulmonary perfusion, a reduction in pulmonary arterial P_{O_2} produced by a decrease in the magnitude of a left-to-right intracardiac shunt

would result in an enhancement of the P_{O_2} gradient from lung gas to the afferent end of the pulmonary capillaries. While this mechanism is a viable alternative to an increase in pulmonary blood flow for enhancing systemic arterial oxygenation, there are no data from this or any other study to substantiate this possibility. Unfortunately, intracardiac shunts conventionally are assessed either by radioactive microsphere distribution (see Heisler and Glass 1985) or by interrupted blood sampling for O_2 saturation determination (see Burggren and Shelton 1979). Neither of these approaches would be likely to reveal clearly any transient adjustments in intracardiac shunting and pulmonary arterial P_{O_2}. Thus, the assessment of the role of changes in intracardiac shunting during the dive, in addition to that of increases in pulmonary perfusion, must await further experimentation involving continuous rather than discontinuous physiological measurements.

The second question posed above involves the relative physiological advantages/disadvantages of each of the two observed patterns of arterial P_{O_2} change during diving. In the presence of the progressive decline in systemic arterial P_{O_2} evident in the first pattern, bulk O_2 unloading in the systemic tissues will also progressively fall unless there is either a proportionate increase in systemic capillary flow or a decrease in tissue P_{O_2}. The ability to maintain systemic arterial O_2 at very high levels throughout quite long dives (the second pattern depicted in figs. 5–7) must confer considerable advantage in supporting aerobic metabolism throughout the dive without necessitating increased capillary flow or decreased tissue P_{O_2}.

Why do *Chelodina* show arterial O_2 homeostasis during less than one-half of their dives, rather than during all of them? One explanation might revolve around experimental disturbance. The sea snake *Achrochordus granulatus* shows a similar pattern of pulmonary blood flow changes during very long quiescent dives, but only if there has been 2–3 d of postsurgical recovery after implantation of electromagnetic flow probes (H. Lillywhite, unpublished). Thus, at least in this snake, disturbance is one factor affecting diving patterns. Yet in our present experiments with *Chelodina,* pulsed pulmonary arterial flow and arterial O_2 homeostasis were not correlated with duration of dive, degree of underwater activity, or length of postsurgical recovery. The difference between *Chelodina* and *Achrochordus* may be simply a species difference, or it may relate to the fact that the impedance technique used to monitor blood flow in the present study is much less invasive than the use of electromagnetic flow probes. We are thus inclined to look for a physiological answer to the question of why arterial O_2 homeostasis does not occur in every dive.

To pulse blood through the lungs in an intermittent fashion at high flow rate, which appears to be an integral part of maintaining arterial O_2 homeostasis, might possibly have a greater metabolic cost than perfusing the lungs

at a slower, more constant rate. Burggren (1987) has argued previously that, in the short term, the overall metabolic cost of blood circulation in general and of intracardiac shunting in particular is only a very small fraction of the total energy budget of freshwater turtles. Yet energetic savings of even fractions of a percent of an animal's total metabolism may have long-term importance to its survival and the ultimate evolutionary success of the species. Thus, while the ability to maintain a constant high arterial O_2 saturation by pulsing blood flow during diving is of undeniable physiological importance to *Chelodina,* it may not be energetically appropriate to exhibit this perfusion pattern under every circumstance during every dive. The long-term energetics of blood flow in reptiles needs further study.

Another explanation for why pulsed pulmonary blood flow might not occur during every dive involves pulmonary fluid balance. Frequent repeated large increases in blood flow to the lungs during every dive could result in the accumulation of excessive plasma filtrate in the pulmonary interstitia and lymphatics (Burggren 1982, 1987; Smits 1989). *Chelodina* may thus need to balance the benefits of maintaining arterial O_2 homeostasis with the need to reduce plasma filtration in the lungs.

Finally, it is important to consider how widespread arterial O_2 homeostasis might be among diving reptiles. This study is the first to search specifically for this phenomenon, as opposed to observing O_2 homeostasis during the course of experiments designed for other purposes. Our general knowledge of reptilian hemodynamics and the mechanisms for changing lung blood flow and gas exchange, along with anecdotal observations mentioned earlier, collectively indicate that the potential for systemic arterial O_2 homeostasis during diving exists in many intermittently breathing reptiles. It has been widely assumed that measurements of blood gases have been made on undisturbed animals acting exactly as they do in the wild and thus that the steady decline in arterial O_2 during diving is the normal pattern. Nonetheless, our observation on numerous occasions of arterial O_2 homeostasis during voluntary diving in *C. longicollis* may have been a direct result of making measurements on minimally disturbed animals by relatively nondisruptive techniques. Until additional studies on other species have been completed with the greatest care to minimize disturbance, it remains possible that arterial O_2 homeostasis is common not only in *Chelodina* but in other diving reptiles and may even be the more typical pattern of diving. Certainly, the potential in reptiles to dive for long periods without suffering the repercussions of diminishing arterial oxygenation warrants much further research.

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Literature Cited

Boutilier, R. G., and G. Shelton. 1986. The effects of forced and voluntary diving on ventilation, blood gases and pH in the aquatic amphibian, *Xenopus laevis*. J. Exp. Biol. 122:209–222.

Burggren, W. W. 1982. Pulmonary plasma filtration in the turtle: a wet vertebrate lung? Science 215:77–78.

———. 1985. Hemodynamics and regulation of cardiovascular shunts in reptiles. Pages 121–142 in K. Johansen and W. Burggren, eds. Cardiovascular shunts: phylogenetic, ontogenetic and clinical aspects. Munksgaard, Copenhagen.

———. 1987. Form and function in reptilian circulations. Am. Zool. 27:5–19.

———. 1988. Cardiovascular responses to diving and their relation to lung and blood oxygen stores in vertebrates. Can. J. Zool. 66:20–28.

———. 1989. Lung structure and function: amphibians. Pages 153–192 in S. C. Wood, ed. Comparative pulmonary physiology: current concepts. Dekker, New York.

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.

Burggren, W. W., C. E. W. Hahn, and P. Foex. 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. 58A:185–191.

Burggren, W. W., and G. Shelton. 1979. Gas exchange and transport during intermittent breathing in chelonian reptiles. J. Exp. Biol. 82:75–92.

Elsner, R., and B. Gooden. 1983. Diving and asphyxia. Cambridge University Press, New York.

Heisler, N., and M. L. Glass. 1985. Mechanisms and regulation of central vascular shunts in reptiles. Pages 334–347 in K. Johansen and W. Burggren, eds. Cardiovascular shunts: phylogenetic, ontogenetic and clinical aspects. Munksgaard, Copenhagen.

Lillywhite, H. B., and J. A. Donald. 1987. Neurovascular structure and function in the elongate lung of an aquatic snake. Physiologist 30:190.

Perry, S. F. 1989. Lung structure and function: reptiles. Pages 193–236 in S. C. Wood, ed. Comparative pulmonary physiology: current concepts. Dekker, New York.

Randall, D. J., W. W. Burggren, A. P. Farrell, and M. S. Haswell. 1980. Evolution of air breathing in vertebrates. Cambridge University Press, New York.

SHELTON, G. 1976. Gas exchange, pulmonary blood supply, and the partially divided amphibian heart. Pages 247–256 in P. SPENCER DAVIES, ed. Perspectives in experimental biology. Vol. 1. Pergamon, Oxford.

———. 1985. Functional and evolutionary significance of cardiovascular shunts in the Amphibia. Pages 100–116 in K. JOHANSEN and W. BURGGREN, eds. Cardiovascular shunts: phylogenetic, ontogenetic and clinical aspects. Munksgaard, Copenhagen.

SHELTON, G., and R. G. BOUTILIER. 1982. Apnoea in amphibians and reptiles. J. Exp. Biol. 100:245–274.

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

SMITS, A. W. 1989. Fluid balance in vertebrate lungs. Pages 503–538 in S. C. WOOD, ed. Comparative pulmonary physiology: current concepts. Dekker, New York.

WHITE, F. N. 1976. Circulation. Pages 275–334 in C. GANS and W. R DAWSON, eds. Biology of the Reptilia. Vol. 5. Academic Press, New York.

———. 1985. Role of intracardiac shunts in pulmonary gas exchange in chelonian reptiles. Pages 296–305 in K. JOHANSEN and W. BURGGREN, eds. Cardiovascular shunts: phylogenetic, ontogenetic and clinical aspects. Munksgaard, Copenhagen.