

The Intracardiac Shunt as a Source of Myocardial Oxygen in a Turtle, *Trachemys scripta*¹

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SYNOPSIS. The functional significance of many features of the reptilian cardiopulmonary system remains unknown; particularly the importance of cardiac shunts. One hypothesis for a physiological function for shunts is that they play a role in myocardial oxygenation and are therefore important when cardiac work is elevated. In this study we examined cardiac function by monitoring electrocardiograms in red-eared slider turtles (*Trachemys scripta*) with a reduced myocardial oxygen supply. Exposing the animals to a hypoxic gas mixture reduced oxygen levels in the pulmonary venous return. When cardiac work was elevated during hypoxia, the electrocardiogram changed in a manner consistent with myocardial hypoxia, suggesting enrichment of the luminal blood with oxygen by the intracardiac shunt facilitates cardiac performance.

INTRODUCTION

Biologists have been interested in the vertebrate cardiovascular system for hundreds of years (Panizza, 1833; Griel, 1903; Axelsson and Franklin, 1996; reviewed in Hicks, 1998); yet there remain numerous features of this system with obscure functions. This is particularly true of the complex cardiovascular system of reptiles. Because the ventricles of reptiles are not completely subdivided into two chambers (with the exception of crocodylians and birds), mixing of oxygen-rich and oxygen-poor blood can occur within the ventricle. When blood is shunted from the left side of the ventricle to the right side of the ventricle, oxygen-rich pulmonary venous blood bypasses the body and is returned to the lung. This blood flow pattern is known as a left-to-right (L-R) shunt (Fig. 1A). Conversely, a right-to-left (R-L) shunt occurs when oxygen-poor systemic venous blood bypasses the pulmonary circulation and is returned to the body. Both birds and mammals evolved a circulatory system that completely separates oxygen-rich and oxygen-poor blood.

Historically, the avian and mammalian cardiovascular systems were considered to be more efficient than the reptilian system (Foxon, 1955). However, when it became clear that the type and degree of cardiac shunting varies with the physiological state of the animal, the idea that shunts are adaptive became credible. Shunts have been hypothesized to confer a number of physiological advantages (Table 1) but data supporting or refuting many of these hypotheses are scarce.

One hypothesis for the functional significance of the L-R intracardiac shunt is that it oxygenates the heart (Farmer, 1997, 1999). In mammals and birds, the heart is oxygenated by a coronary circulation, an extensive network of arteries, capillaries, and veins. In contrast,

the hearts of most fishes, amphibians, and reptiles are primarily avascular. The cardiac tissue is a mesh of intercommunicating spaces (lacunae) and numerous strands or beams (trabeculae) of heart muscle (myocytes) encased by connective tissue, the epicardium. As the heart beats, blood flows to and fro through the lacunae. This tissue resembles a sponge and is called "spongy myocardium." The exchange of respiratory gases and nutrients occurs directly across the plasma membranes of the myocytes with blood contained within the lacunae (reviewed in Farmer, 1999). When coronary supported myocardium is found in reptiles, it is generally a thin layer of more compact fibers that form a shell just proximal to the epicardium. For example, Brady and Dubkin (1964) report that in a turtle (*Chrysemys elegans*) a distal shell of muscle, making up about 10% of the mass of the ventricle, is supplied oxygen through a coronary circulation. The remaining myocytes receive oxygen from luminal blood.

The L-R intracardiac shunt carries oxygen-rich blood into regions of the heart that would otherwise be oxygen-poor (Hicks and Malvin, 1995; Hicks *et al.*, 1996). Thus, this shunt may be important for myocardial oxygenation. This hypothesis predicts that the L-R shunt will occur when the cardiac workload is elevated, since myocardial oxygen demands are greater when the heart is working hard than when the heart is at rest (Bing *et al.*, 1972; Farrell *et al.*, 1994, but see Jackson *et al.*, 1995). In reptiles, cardiac workloads are not only elevated during exercise and immediately during recovery from exercise but also during periods of ventilation. Reptiles generally are intermittent lung breathers that exhibit a pronounced cardiorespiratory synchrony (reviewed in Hicks, 1998). When at rest during periods of apnea, these animals generally exhibit bradycardia and reduced cardiac output. During ventilation, there is generally an increase in heart rate and an elevation in cardiac output (Burggren, 1975; Shelton and Burggren, 1976; reviewed in Hicks, 1998).

As predicted by the hypothesis that a L-R shunt provides oxygen to the heart, previous research indicates that a large L-R shunt develops during exercise and during periods of ventilation (Shelton and Burggren,

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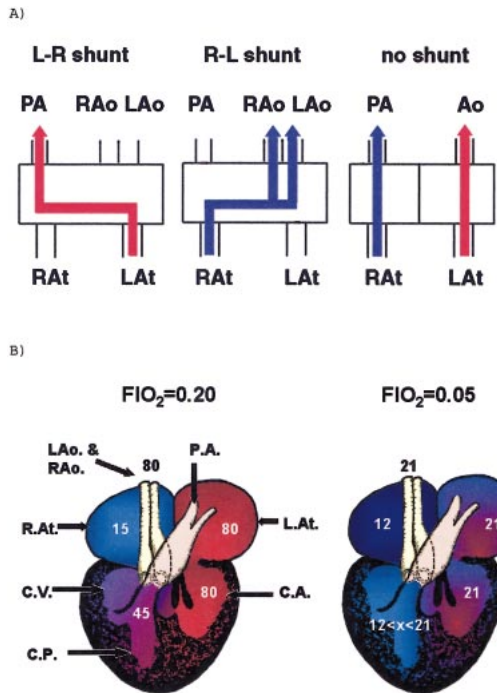


FIG. 1. Cardiac blood flow patterns. A) A left-to-right (L-R) shunt occurs when oxygen-rich pulmonary venous blood entering the left atrium (LA) is shunted to the right side of the ventricle to be ejected into the pulmonary artery (PA). A right-to-left (R-L) shunt occurs when oxygen-poor systemic venous blood entering the right atrium (RA) is shunted over into the left side of the ventricle and ejected into the systemic aorta (LAo, RAo). Complete subdivision of the ventricle (e.g., mammals and birds) results in complete separation of oxygen-rich and oxygen-poor blood. B) Schematic of a turtle heart during a L-R shunt illustrating the experimental design, which aimed to expose the vigorously working heart to blood that contained a partial pressure of oxygen typical of the mixed systemic venous return of exercising turtles. During a left-to-right (L-R) shunt, oxygen-rich blood that is in the cavum arteriosum (C.A.) passes through the cavum venosum (C.V) and into the cavum pulmonale (C.P) to mix with the oxygen-poor blood of the systemic venous return. This admixture is then ejected into the pulmonary artery (P.A.). Blood from the cavum arteriosum is ejected into the right and left aortas (RAo and LAo respectively). Estimates of the partial pressure of oxygen within the heart during a L-R shunt are superimposed on the respective chambers. While exercising and breathing a gas with a fraction of inspired oxygen of 0.20 ($FI_{O_2} = 0.20$), we found that the partial pressure of oxygen of the mixed systemic venous return (P_{VO_2}) was 15 ± 4.7 torr. Previous studies have shown that during a L-R shunt, the partial pressure of oxygen in arterial blood (P_{AO_2}) flowing in the aortas is the same as left atrial blood, and is around 80 torr (Ishimatsu *et al.*, 1996). Furthermore, pulmonary arterial blood has been measured to have a PO_2 as much as 30 torr greater than mixed systemic venous blood (Ishimatsu *et al.*, 1996). The right side of the panel illustrates approximations of blood gases within the heart when the turtles swam with $FI_{O_2} = 0.05$. Mixed P_{VO_2} was measured to be 12 ± 1.4 torr and P_{AO_2} was 21 ± 2.8 torr. Hence the partial pressure of oxygen of blood in the ventricle ranged between 12 and 21 torr. Illustration is based in part on an unpublished drawing by F.N. White. Blue represents oxygen-poor blood, red oxygen-rich blood.

1976; West *et al.*, 1992; Hicks and Krosniunas, 1996; Wang and Hicks, 1996; Wang *et al.*, 1997; Hicks and Wang, 1998). Although consistent with the hypothesis, these data do not demonstrate that a L-R shunt is serving to oxygenate the heart. The shunt may occur at

these times for different reasons. For example, it has been shown that development of a L-R shunt is often associated with a reduction in R-L shunt, thus improving systemic oxygen transport by increasing systemic arterial saturation (Hicks, 1994). Consequently, more data are needed to determine the importance of L-R shunting for myocardial oxygenation. The following study was undertaken to assess cardiac performance of red-eared slider turtles deprived of elevated oxygen levels in the heart during periods of increased oxygen demand.

MATERIALS AND METHODS

Experimental design

A direct test of our hypothesis would be to completely subdivide the ventricle, preventing a L-R intracardiac shunt. Thus, it would be possible to determine whether myocardial function is reduced during periods of increased oxygen demands when oxygen-rich blood is prevented from shunting into the right side of the ventricle. However, subdividing the reptilian heart is not feasible. An alternative approach would be to reduce the magnitude of the L-R shunt by manipulating the vascular resistance in the pulmonary and systemic circulation, either pharmacologically or mechanically (Hicks *et al.*, 1996). However, this approach may confound the results by affecting the afterload or contractility of the heart. Hence, we used an indirect approach to examine the hypothesis that L-R shunts provide oxygen necessary for cardiac function under elevated demands by addressing the following question: If the ventricle were only exposed to blood that had a partial pressure of oxygen (PO_2) similar to that of mixed systemic venous return during exercise, would cardiac function be affected?

We chose to address this question in turtles because more is known about when and how they shunt than for other reptiles. However, at low workloads the hearts of turtles are well known for their tolerance of anoxia (Jackson, 1987; Wasser *et al.*, 1990; Jackson *et al.*, 1991, 1995) and it is possible that the myocardium could function normally without any enrichment of blood oxygen from L-R shunts. Hence we investigated cardiac performance by monitoring the electrocardiogram (ECG) when the vigorously working heart was subjected to blood with a reduced PO_2 .

All experiments were conducted at 23°C. Furthermore, we carried out the experiments in several steps. First, we determined the pH and PO_2 in the mixed systemic venous return in resting and in swimming turtles while normoxic gas passed through the air-hole of the swimming chamber. Then we reduced the percentage of oxygen in gas of the air-hole and swam the turtles. We decreased the fraction of inspired oxygen (FI_{O_2}) so that the PO_2 of pulmonary venous blood approached values that were similar to those measured in the systemic venous return of normoxic animals (Fig. 1B). We estimated the PO_2 of pulmonary venous blood by measuring the PO_2 of the blood in the carotid artery.

TABLE 1. *Hypothesized functions of reptilian shunts.*

Function	Direction of shunt	Reference
Facilitates warming	R-L	(Tucker, 1966; Baker and White, 1970)
Facilitates gastric HCl secretion following a meal	R-L	(Webb, 1979; Jones and Shelton, 1993)
Reduces plasma filtration into the lungs	R-L	(Burggren, 1982)
Reduces CO ₂ flux into the lungs	R-L	(White, 1985)
Saves cardiac energy	R-L	(Burggren, 1987)
Meters lung oxygen stores	R-L	(Burggren <i>et al.</i> , 1989; Grigg, 1989)
Triggers hypometabolism	R-L	(Hicks and Wang, 1999)
Facilitates recovery from an extracellular acidosis	R-L	(Farmer, 2000; Farmer and Carrier, 2000)
Facilitates CO ₂ elimination into the lung	L-R*	(Ackerman and White, 1979; White, 1985)
Minimizes ventilation perfusion mismatching	L-R*	(Wood, 1984; West <i>et al.</i> , 1992)
Improves systemic O ₂ transport	L-R*	(Hicks, 1994)
Myocardial oxygenation	L-R*	(Farmer, 1997)

*Does not apply to crocodylians.

During a L-R shunt, the PO₂ of blood in these vessels is approximately the same (Ishimatsu *et al.*, 1996). There is a slight decrease in the oxygen levels of blood in the carotid compared to the blood entering the heart from the pulmonary vein due to the fact that some oxygen has been consumed by the myocytes. Nevertheless, by decreasing the oxygen levels in pulmonary venous blood, we ensured that the myocardium was bathed with blood containing a level of oxygen similar to that normally occurring in the systemic venous blood during exercise, even though the magnitude of the L-R shunt increased during activity. The sensitivity of the myocardium to this range of PO₂s was assessed with ECGs. These experiments are referred to as series 1: Blood gases and electrocardiograms.

We then investigated the relationship between the ECG patterns and cardiac performance by measuring cardiac output and ECGs simultaneously when subjecting turtles to the same experimental regime. In addition, we were able to obtain arterial pressure measurements from three of these animals to examine cardiac power. These experiments are referred to as series 2: Blood flow and electrocardiograms.

In summary, our experimental design aimed to examine the ECG of a vigorously working heart while the heart was supplied with oxygen by blood that had a partial pressure of oxygen similar to what is found in the mixed systemic venous return of exercising turtles.

Animals. Red-eared sliders, *Trachemys scripta*, of mixed sex weighing 1.2 ± 0.5 kg were obtained from a commercial dealer (Lemberger Inc., Oshkosh, Wisc., USA). The animals were housed in large aquariums equipped with dry basking areas and heat lamps. They were fed a diet of live goldfish and kept under a 12:12 L:D photoperiod.

Swimming chamber. The swimming chamber ($61 \times 27.7 \times 15.2$ cm) consisted of a rectangular box. Four sides were constructed of acrylic and two ends of plastic grid. This chamber was set in a water-filled flume. The top of the swimming chamber contained an elevated acrylic "air-hole" ($2.5 \times 15.0 \times 2.5$ cm). The

air-hole had a port on each end that allowed gases to pass through the chamber.

Data acquisition. All electrical signals were converted from analog to digital by an MP100 AD converter (Biopack System, Goleta, Calif., USA) and recorded on a Macintosh computer using AcqKnowledge software (Biopack System, Goleta, Calif., USA).

SERIES 1: BLOOD GASES AND ELECTROCARDIOGRAMS

Turtles ($n = 5$) of mixed sex (0.9 ± 0.4 kg) underwent surgery for blood collection and electrode placement. However, not all of the catheters were patent during the experiments. ECGs were obtained from all five animals.

Surgery

Turtles were anesthetized with halothane and the neck injected with Lidocain. The carotid and jugular were catheterized (PE 50 tubing) and the jugular catheter was slid into the vena cava to obtain samples of the mixed systemic venous return. Placements of the catheters were confirmed post-mortem. Catheters were flushed twice daily with heparinized saline (200 iu). The surgeries required 20 min and the animals recovered for at least 24 hr prior to experiments. Post-surgical analgesics and antibiotics were administered.

Electrocardiogram lead placement

Stainless steel nuts and bolts secured an electrode wire in small holes (1.6 mm) that were drilled in the shell at the junction of the plastron and carapace over each leg. Silicone insulated the bolt and wire. The three standard limb leads were recorded in the following way: lead 1 between the right and left forelimbs; lead 2 between the right and left hindlimbs; lead 3 across the left fore and hindlimbs. A fourth signal between the left hindlimb and an electrode placed ventrally over the center of the heart (analogous to the original precordial lead used on humans and referred to in this paper as lead 4 or as P). Additionally, a non-standard lead was observed across the left forelimb and right hindlimb. The signals were filtered with a

bandpass filter (1 Hz to 3 kHz FWHM) and a notch filter (60 Hz) and amplified 5,000 fold (Grass P5, Quincy, Mass., USA).

Experimental protocol. A turtle was placed in the flume and given one hour to relax. Then ECGs were recorded during a period of ventilation and mixed systemic venous blood was drawn into a chilled glass syringe and analyzed immediately for PO_2 and pH (Radiometer BMs MK2 blood gas analysis system; Copenhagen, Denmark). To prevent excessive blood withdrawal, no arterial samples were taken at this time. Ventilation was monitored visually. The turtle was then forced to swim while normoxic gas ($FIO_2 = 0.20$) flowed through the breathing chamber. Five minutes into a 10 min bout of exercise, both venous and arterial blood-samples were drawn and analyzed. The animal was given 20 min to recover. The chamber was then flushed with hypoxic gas, either $FIO_2 = 0.10$ or 0.05 . After one hour of resting in the hypoxic gas mixture, ECGs were recorded during ventilation and both venous and arterial blood samples taken. The animal then swam while exposed to the hypoxic gas and another set of blood samples was taken. The animals recovered from exercise for 8–10 min while the air-chamber remained hypoxic, then they recovered an additional 20 min while normoxic gas flowed through the chamber.

SERIES 2: BLOOD FLOW AND ELECTROCARDIOGRAMS

Surgery

Turtles ($n = 5$) were anesthetized by ventilating their lungs (SAR-830 ventilator, CWE Inc., Ardmore, Penn., USA) with a 4% halothane gas mixture. Upon reaching anesthesia (no response to pinching of the limbs) the halothane concentration was reduced to 1%.

A rectangular opening (4×5 cm) cut into the plastron exposed the cardiovascular system. Ultrasonic flow probes (2R Transonic Systems, Inc., Ithaca, N.Y., USA) were placed around the following vessels: the left pulmonary artery (LPA), the left aorta (LAo), and the branch of the right aorta (RRAo) distal to the subclavian and carotid branching. This last site was selected due to its accessibility. It was used to estimate blood flow in the entire right aorta (RAo) in the following manner: $Q_{RAo} = 1.85 \times Q_{RRAo}$. This estimate has been found to be valid for different physiological states (Comeau and Hicks, 1994) and has been studied for anaesthetized and recovered animals during both apnea and during ventilation in room air, as well as with anoxic animals (Wang and Hicks, 1996).

A small vessel branching from the left subclavian was occlusively cannulated (PE 50 tubing) for arterial blood pressure measurements. Post mortem, the cardiac ventricles were removed and weighed wet.

Electrocardiogram lead placement

Short sections (2 mm) of electrode wires (As 765-40 Cooner Wire, Chatworth, Calif., USA) were cleared of insulation and placed with a 25 gauge needle into the pericardium over the left and right atria and over

TABLE 2. *Blood gases.*

FIO_2	PVO_2	PaO_2	pH	n
0.20 rest	29 ± 8.8		7.59 ± 0.060	5
0.20 exercise	15 ± 4.7		7.51 ± 0.072	5
0.10 rest	22 ± 2.4		7.69 ± 0.081	4
0.10 rest		33 ± 9.4	7.65 ± 0.099	5
0.10 exercise	17 ± 0.6		7.55 ± 0.081	4
0.10 exercise		31 ± 11.4	7.69 ± 0.055	4
0.05 rest	16 ± 3.1		7.68 ± 0.022	3
0.05 rest		23 ± 3.2	7.74 ± 0.024	3
0.05 exercise	12 ± 1.4		7.63 ± 0.079	2
0.05 exercise		21 ± 2.8	7.77 ± 0.029	3

FIO_2 , the fraction of oxygen in the inspired gas; PVO_2 , the partial pressure of oxygen of the mixed systemic venous return; PaO_2 , partial pressure of oxygen in systemic arterial blood (drawn from the carotid); n, number of animals studied.

the apical ends of the left and right sides of the ventricle. An additional electrode was placed over the center of the ventricle. A small drop of Nexaband glue held the electrodes in place. The ECG wires were connected to shielded wire (5/30-4046SJ) and the electrical signals from these leads represented an Einthoven triangular arrangement. Lead 1 refers to the signal obtained when recording from the wires connected over the left and right atria, lead 2 from the right atrium and left apex, lead 3 across the left atrium and left apex, and lead 4 across the center of the ventricle and the left apex (analogous to the original precordial lead). The signals were processed as described previously. The excised piece of plastron was glued into place with epoxy. Antibiotics (Baytril 2.5 mg/kg, daily) and analgesic (0.5 mg/kg Flumeglumine, daily) were administered intramuscularly after the surgery. Animals were given seven to ten days to recover from the surgery.

Calculation of cardiac power

Ventricular systemic power output, VSPO (mW), was calculated per gram of wet ventricular mass during the last minute of the exercise period by multiplying the mean systemic blood pressure by the systemic blood flow (LAo + RAo flow).

Protocol

The same protocol was used and is described above in the section entitled "Blood gas and electrocardiogram."

RESULTS

Series 1: Blood gases

Blood PO_2 and pH are summarized in Table 2. The ECGs recorded during the blood gas measurements were consistent with the ECGs obtained from animals studied during the blood flow measurements. Hence these results were pooled.

Series 2: Blood flow

To control for differences in the ECG that might be accounted for by respiratory state (Burggren, 1978),

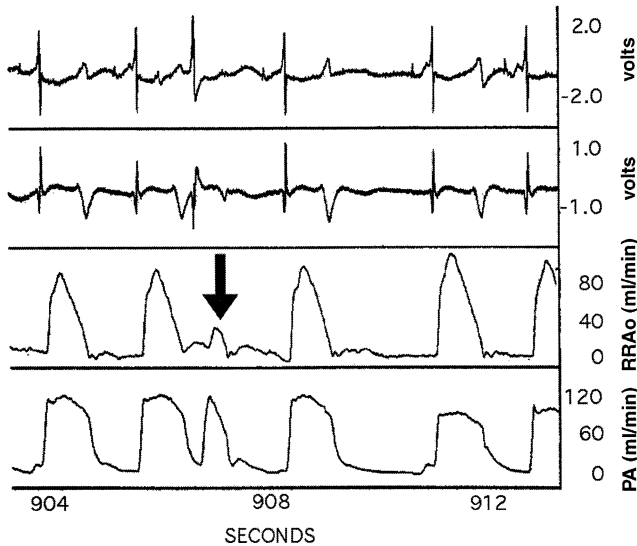


FIG. 2. The ECG from pericardial leads 1 and 4 (first and second traces respectively) and blood flow in the right branch of the right aorta (RRAo) and pulmonary artery (PA) (third and fourth traces respectively) during ventilation both before and after exercise with a fraction of inspired oxygen of 0.05 ($FI_{O_2} = 0.05$). The arrow marks an anomalous QRS complex that corresponded to a reduced stroke volume.

periods of ventilation were examined in this study before and immediately after exercise. To reduce artifacts caused by swimming motions, ECGs were recorded immediately after exercise rather than during exercise. After exercising while breathing hypoxic gases, the ECGs were abnormal compared to those recorded before and after exercise with $FI_{O_2} = 0.20$. Coincident with the onset of these electrical anomalies were signs of myocardial dysfunction, such as ventricular depolarizations that did not result in sufficient contraction to cause ejection, or resulted in reduced stroke volumes (Fig. 2). Post exercise function remained impaired and the ECG abnormal until FI_{O_2} returned to normal (Fig. 3). At this time the ECG returned to a pattern similar to that observed before exercise. The top trace of Figure 3 shows an ECG recorded from lead 1 during ventilation ($FI_{O_2} = 0.20$) before any exercise was undertaken. During ventilation after exercise with $FI_{O_2} = 0.05$, the T wave was inverted and the PR interval increased. These electrical anomalies remained until FI_{O_2} increased to approximately 0.12, at which time the T wave returned to an upright position and the PR interval shortened. Figure 4 illustrates inversion of the T wave on a different animal that is apparent on lead 2 and 3. The left side of the figure shows the ECG during ventilation before exercise and the right side shows the ECG during ventilation after exercise. FI_{O_2} was 0.20 in the top traces and 0.05 in the bottom traces. Inversion of the T wave was seen after exercise with $FI_{O_2} = 0.05$, but not on all the leads.

During and after exercise with FI_{O_2} of 0.10 and 0.05, the hearts also became arrhythmic and the ECG exhibited a pattern consistent with atrial-ventricular

(A-V) block. Figure 5A shows a continuous recording of blood flow in the RRAo that illustrates heart beats that occur in doublets and triplets. Furthermore, changes in the atrial ECG were observed. Figure 5B illustrates multiple P waves without corresponding QRS waves.

Cardiac power ($N = 3$) significantly ($P = 0.048$ one tailed paired t -test) declined from 3.1 ± 0.5 $mW\ gm^{-1}$ ventricle (mean \pm SE) during normoxic exercise to 1.0 ± 0.3 $mW\ gm^{-1}$ ventricle (mean \pm SE) during hypoxic exercise ($FI_{O_2} = 0.05$) (Fig. 6).

DISCUSSION

In general, the demand for myocardial oxygen is a function of how vigorously the heart is working, consequently myocardial oxygen requirements increase during ventilation and during exercise in turtles. It is well established that a net L-R shunt develops during breathing and during activity, when myocardial workload is elevated (Shelton and Burggren, 1976; West *et al.*, 1992; Hicks and Krosniunas, 1996; Wang and Hicks, 1996; Wang *et al.*, 1997). The development of a large L-R shunt can significantly increase the oxygen available to the right side of the heart. For example, a study on anesthetized turtles showed that during periods of L-R shunting, the PO_2 of pulmonary arterial blood can be as much as 30 torr greater than the values found in the right atrium (Ishimatsu *et al.*, 1996). Consequently it is possible that the L-R shunt provides oxygen to the myocardium.

To address the question of whether or not cardiac function is impaired without the L-R shunt is difficult. A direct test of this hypothesis would require surgically subdividing the ventricle, which is not feasible. Consequently the approach of the current study was indirect. Many reptiles, particularly turtles, are well known for their tolerance to hypoxia (Jackson, 1987; Wasser *et al.*, 1990; Jackson *et al.*, 1991, 1995); hence the levels of oxygen found in systemic venous blood may be adequate for sustaining normal cardiac function. Therefore, it was critical to determine the sensitivity of the turtles' hearts to low oxygen.

In resting turtles breathing room air, the PO_2 of the mixed systemic venous blood was 29 ± 8.8 (SD) torr. This is similar to values measured in previous studies (Burggren and Shelton, 1979; Hicks and Wang, 1998). When swimming, the mixed venous PO_2 decreased to 15 ± 4.7 (SD) torr (Fig. 2). During activity, with $FI_{O_2} = 0.05$, mixed systemic venous blood PO_2 was 12 ± 1.4 (SD) torr and the arterial blood was 21 ± 2.8 (SD) torr. During this period, the chambers of the heart contained an oxygen tension in the range of 12 to 21 torr, which encompasses the 15 torr measured in the systemic venous return of turtles swimming with access to normoxic air.

During periods of elevated cardiac work under hypoxic conditions we found several indicators of reduced cardiac performance: (1) electrical anomalies; (2) arrhythmia; (3) bradycardia; (4) decrease in systemic ventricular power.

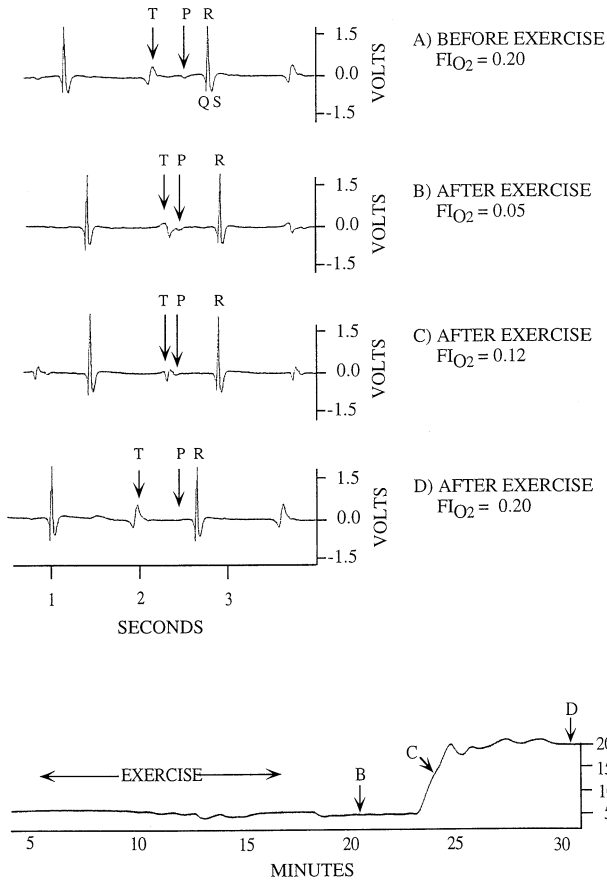


FIG. 3. The ECG during ventilation. The bottom trace shows the level of oxygen flowing through the air-box before, during, and after exercise. This trace also shows several periods selected to illustrate changes that occurred in the ECG after the animal exercised with a fraction of inspired oxygen of 0.05 ($FI_{O_2} = 0.05$; B, C, and D). The traces in the panels marked A through D are segments of an ECG recorded on lead 1. Trace (A) shows several cardiac cycles recorded during ventilation with $FI_{O_2} = 0.20$, before any exercise was performed (not shown in the bottom of the figure). Trace (B) shows the ECG after exercise with $FI_{O_2} = 0.05$; the T wave is inverted and the P-R interval has increased compared to trace (A). Trace (C) shows the ECG when the T wave begins to return to normal. Trace (D) shows the ECG after the animal has been breathing $FI_{O_2} = 0.20$ for about seven minutes. The T wave and the P-R intervals have returned to normal. P is the signal resulting from depolarization of the atria. QRS complex results from depolarization of the ventricle. T is the electrical signal caused from repolarization of the ventricle.

The most sensitive indicator of myocardial hypoxia is electrical anomalies, which can often be detected before any gross malfunction occurs (Marriott, 1983). Although systematic analysis of ECGs during myocardial hypoxia has not been conducted in reptiles, our analysis of the ECG in this study indicated several changes that are consistent with myocardial hypoxia (Marriott, 1983). During the ventilatory period with $FI_{O_2} = 0.20$, pre-exercise and post-exercise ECGs were similar. In contrast, reductions in FI_{O_2} to 0.10 and 0.05 during exercise and immediately following exercise resulted in inversion of the T wave. Inversion of the T wave at $FI_{O_2} = 0.05$ was seen for all the

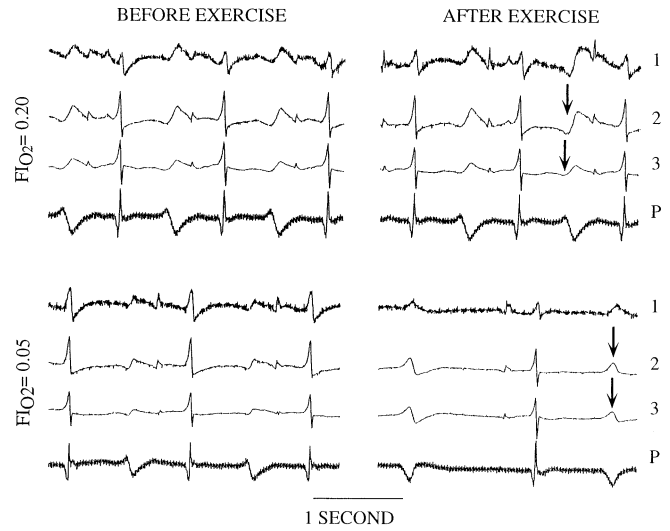


FIG. 4. ECGs recorded on pericardial leads 1, 2, 3, and P during $FI_{O_2} = 0.20$ (top) and 0.05 (bottom). The left side of the figure shows ventilation before exercise and the right side shows ventilation immediately after exercise. The most significant difference in the ECG before and after exercise in $FI_{O_2} = 0.20$ is an increased prominence of the P wave after exercise on lead 1. The T waves are not inverted. However, after exercising with $FI_{O_2} = 0.05$ (the lower traces), the T waves (indicated by the arrows) invert on lead 2 and 3 compared to before exercise and compared to after exercise with $FI_{O_2} = 0.20$. Bradycardia is present both before and after exercise during hypoxic conditions.

animals studied, but was not seen for all the turtles at $FI_{O_2} = 0.10$. This inversion is illustrated in Figures 3 and 4 for two of the turtles. The T wave was not inverted on all of the leads examined (Fig. 4), suggesting that inversion was not due to pericarditis or effusion (Marriott, 1983). At low levels of inspired oxygen ($FI_{O_2} = 0.05$) additional anomalies in the ECG oc-

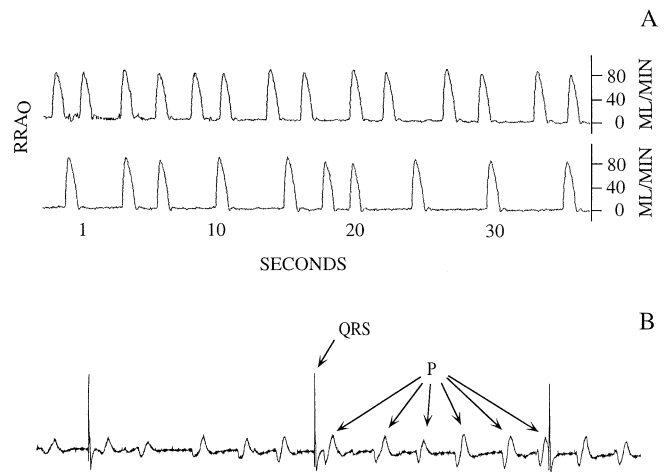


FIG. 5. (A) A pattern of ejection that was seen typically in animals exercising with $FI_{O_2} = 0.10$ or less. The top and bottom traces are continuous in time and illustrate an arrhythmia that tends to form in pairs, trios, and groups of four beats. (B) The ECG from a turtle that exhibited multiple P waves for each QRS complex. On this lead the T waves are small and are masked by the P waves, but they were visible on other leads.

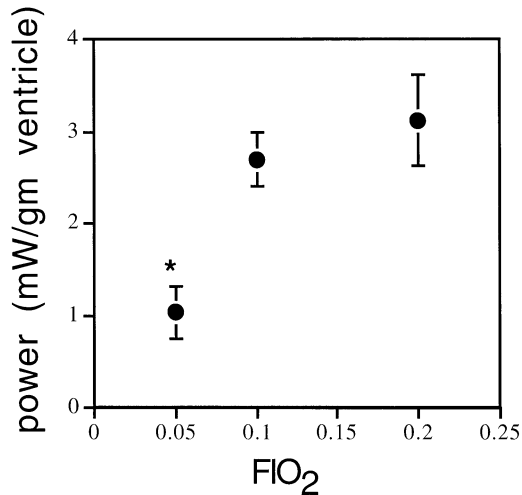


FIG. 6. Preliminary data of cardiac power measurements ($N = 3$; mean \pm SE) made during the last minute of exercise. Power dropped by approximately 2/3 when the animals swam with $FIO_2 = 0.05$ compared to normoxia. * indicates a value significantly different from normoxia ($P \leq 0.05$; one tailed paired t -test).

curred (e.g., appearance or increased prominence of Q waves, ST segment changes).

In addition to changes in ventricular depolarization and repolarization in the ECG, changes in the P-R interval were observed (Figs. 3, 5). In mammals, a gradual lengthening of the P-R interval can result from ischemic heart disease and produces a pattern of ventricular contractions that is characterized by groups of beats, especially pairs, trios, etc. These patterns are known as the "footprints of Wenckebach" and can be used to identify AV block, even when the P-wave is not seen in the ECG (Marriott, 1983). Patterns of pairs and trios that are similar to the footprints of Wenckebach observed in humans were found in turtles breathing hypoxic air (Fig. 5A) and have been observed in perfused turtle hearts subjected to a hypoxic solution (Farmer, personal observation). Furthermore, recordings from pericardial leads show a progressive lengthening in the P-R interval, as well as high grade (advanced) second degree AV block, where a series of P waves occurs without an ensuing T wave (Fig. 5B). This is consistent with an apparent A-V block observed in anoxic isolated turtle heart strips (Jackson, 1987). Finally, these findings in turtles are also consistent with atrial extra-systoles observed in hypoxic carp (Rantin, 1993).

The electrical changes we observed in turtles during hypoxia are consistent and similar to changes seen by other researchers in the ECG of reptiles. For example, T wave amplitude changes have been reported in the tuatara (*Sphenodon punctuatus*), carp (*Cyprinus carpio*), and a turtle (*Trachemys scripta*) (McDonald and Heath, 1971; Glass *et al.*, 1991; Rantin, 1993; Altimiras, 1995). Glass *et al.* (1991) found an increased T wave during aquatic hypoxia in carp and Rantin (1993) reports inversion of the T wave with extreme hypoxia in carp. Changes in ventricular depolarization (the

QRS complex) associated with ventilation (compared to apnea) have been observed in turtles (Burggren, 1978), although Altimiras (1995) did not detect a similar pattern, perhaps due to differences in methodology, but did detect on rare occasions inversion of the T wave.

Alternate explanations. The electrical changes observed in turtles after exercise while breathing hypoxic gas are similar to electrical changes observed in ischemic mammalian hearts and include inversion of the T-wave, heightened T-wave, elongation of the P-R interval, and multiple p waves without a corresponding QRS complex. However, electrocardiography does not always provide a clear-cut diagnosis because similar anomalies can occur for a variety of reasons. For example, massive pulmonary embolism can produce an electrical signature typical of myocardial infarction. Pericarditis, myxedema, hypokalemia, hyperkalemia, hypocalcemia can affect the hearts electrical signals (Marriott, 1983). Thus, our measurements of blood flow and cardiac power were important to verify that the electrical anomalies were indeed a result of the hypoxic treatment and that they were harbingers of impaired cardiac function.

We found that anomalies in the ECG corresponded to problems with ejection (Figs. 2, 5). We also found that hypoxia reduced systemic ventricular power output, which is consistent with reductions of ventricular power reported for anoxic and acidic turtle hearts during heavy workloads (Farrell *et al.*, 1994; Shi and Jackson, 1997; Hicks and Wang, 1998).

In summary, the changes of the ECG that occurred in the turtles when the myocardium was hypoxic (the ventricle contained blood with a PO_2 that ranged between 12 and 21 torr) suggest that maximum power and performance of the heart cannot be maintained on the oxygen available in the mixed systemic venous return of exercising animals. Therefore, the additional oxygen that is washed into the right side of the heart by the L-R shunt appears to contribute to myocardial oxygenation and to maintaining cardiac performance.

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