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The mechanism and function of transient pressure fluctuations occurring in the lungs during diving in the turtle, Trachemys (=Pseudemys); scripta elegans

Henry Lee Bermudez
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Bermudez, Henry Lee, M.S.

University of Nevada, Las Vegas, 1994

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THE MECHANISM AND FUNCTION OF TRANSIENT PRESSURE FLUCTUATIONS OCCURRING IN THE LUNGS DURING DIVING IN THE TURTLE, TRACHEMYS [=PSEUDEMYS] SCRIPTA ELEGANS.

by

Henry Lee Bermudez

A thesis submitted in partial fulfillment of the requirements for the degree of

Master of Science

in

Biology

Department of Biology
University of Nevada, Las Vegas
May 1994

THE MECHANISM AND FUNCTION OF TRANSIENT PRESSURE FLUCTUATIONS OCCURRING IN THE LUNGS DURING DIVING IN THE TURTLE, TRACHEMYS SCRIPTA ELEGANS.

The thesis of Henry Lee Bermudez for the degree of Master in Biology is approved.

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May 1994

ABSTRACT

Recurring, brief fluctuations in lung pressure, refered to as Lung Pressure Transiets (LPTs), lasting approximately 5-6 seconds and with an amplitude of +0.25-2.0 mmHg were measured in nearly 30% of all voluntary dives in the aquatic turtle, *Trachemys scripta elegans*. LPTs were studied to determine if they could enhance pulmonary gas exchange. Skeletal muscle is the primary mechanism responsible for creating these pressure fluctuations, based on their persistence following inactivation of smooth muscle by atropine, a muscarinic blocking agent. Gas distribution experiments using helium injected into the lungs indicated that pulmonary gas distribution within a single lung and between lungs occurs nearly twice as rapidly in dives in which LPTs occur as in dives lacking LPTs. Collectively, these data indicate that LPTs reflect actual contraction of the lung(s) that lead to volume changes. Pulmonary gas exchange during diving is enhanced by LPTs due to stirring of stagnant pockets of lung gas and by the disruption of boundary layers present adjacent to respiratory gas exchange surfaces.

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Dedicated to my mother and father
LINDA AND ENRIQUE D. BERMUDEZ

and to the memory of Dr. R. Keith Dupre
who did not live to see this accomplishment

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CHAPTER 1

INTRODUCTION

In the course of their evolution, many members of the vertebrate classes have had to adapt to gas exchange within the water and subsequently to gas exchange in the open air. These animals have shown an extensive range of respiratory mechanisms. The impacts of their evolution can be seen today in all modern amphibians and reptiles. Today biologists and physiologists study the Reptilia to better understand physiological mechanisms in the more derived animals of present day.

Examination of the ventilatory mechanisms in reptiles allows for a better general understanding of all vertebrate respiratory systems. In even the simplest respiratory systems, one can identify and describe the "pump" that ventilates some form of "exchanger". The exchanger acts as the interface between the organism and its internal environment (Perry, 1983), and is the site of gas exchange. The concept behind these two mechanisms seems quite simple, yet modern day reptiles have evolved some complex and unique respiratory advancements

specifically related to their aquatic or semi-aquatic lifestyles. One particular Order, the Chelonia, stands out amongst the reptiles. Presence of an outer, inflexible shell has required respiratory mechanisms unlike those of other reptiles. One excellent example is the principle behind the mechanism of pulmonary ventilation. The lungs of a turtle can best be envisioned as a balloon encased within a solid box. Special adaptations have evolved with regard to the skeletal girdle and surrounding musculature. Both play an important role in breathing and to an extent in assisting gas exchange. Many chelonians are divers. The act of diving in itself is a remarkable process, and the physiological adaptations associated with diving are complex. *Trachemy scripta elegans* is an aquatic pond turtle that has been under study since the late eighteenth century. It has intrigued researchers not only with its fascinating physiology, but its confusing nomenclature (Appendix One).

I. Review of Diving Physiology

To understand the role of the respiratory system, including the lungs, in diving reptiles, I will briefly review: (A) lung morphology and breathing mechanisms, (B) the role of the lung as a gas source and sink, (C)

ventilation and the transfer of gas across the lung during apneic periods, (D) relationship between ventilation and lung perfusion, (E) the control of buoyancy, (F) pulmonary circulation, and (G) autorhythmic contraction of the lung.

A. *Lung Morphology and Breathing Mechanism*

Air enters the pharynx by means of the glottis. This structure is composed of two (skeletal) muscular flaps and is located on the superficial surface of the pharynx posterior to the tongue. The trachea branches into a left and right primary bronchus at the base of the neck and subsequently enters each lung at the hilus. Upon piercing the lungs each bronchus immediately divides into numerous bronchioles. The trachea and first few divisions of the bronchi are supported by rings of hyaline cartilage. Respiratory morphology has been well documented in the turtle, *Trachemys* [=*Pseudemys*] *scripta elegans* (Burggren *et al.*, 1978; Perry, 1978, 1983). The lungs of this species of turtle are classified as multicameral (Perry, 1983) because they are subdivided into various chambers that are bilaterally symmetrical on either side. The lungs, which lack an extensive bronchial system, adhere closely to various regions of the carapace near the vertebral column. The outer wall of the lung is primarily composed of collagen fibers, elastic connective tissue,

and some smooth muscle. The outer wall is also laden with large visible blood vessels, while the most anterior regions of the lung contain a high concentration of capillary beds. In the tegu lizard (*Tupinambis nigropunctatus*) and the monitor (*Varanus exanthematicus*) nonvascular smooth muscle occurs in superficial portions of the partitions of the lungs, yet no function for this muscle has been determined (Perry, 1983). Internally each investment is constructed with a framework resembling a honey-comb. This structure is formed by trabeculae which are composed of smooth muscle and elastic connective tissue (Perry, 1972). This polygonal network has been termed edicular (Dunker, 1981; Perry, 1983). Internally this lung lacks broad alveolization, and the lumen of each chamber is open and tissue free.

There is a large band of skeletal muscle which rests directly above the lung (striatum pulmonale) as well as several sets of antagonistic (skeletal) muscles (*M. serratus magnus, transversus abdominus,* and *obliquus abdominus)* which span the flank cavities adjacent to the limbs (Perry, 1983). Anteriorly, this skeletal muscle band adheres closely to the lung wall. McCutcheon (1943), conducted experiments which revealed that contraction of these muscles (*serratus magnus* and *oblique abdominus*) causes an increase in the volume of the body cavity in

chelonians. There is a decrease in pressure on the lungs and this forces the lungs to expand, hence beginning the process of inspiration. McCutcheon (1943) also cites the antagonistic muscle of the posterior flank cavity (*diaphragmatics* and *transverse abdominus*) as responsible for creating the pressure changes required for expiration. Gans and Hughs (1967) also cited these specific muscle groups as the "respiratory muscles".

B. *The Role of the Lung as a Gas Source and Sink*

Several distinct mechanisms and adaptations for the storage of oxygen have been reviewed and include: the development of larger lungs (Perry, 1978), and modifications to myoglobin and hemoglobin in relation to oxygen binding affinities (Dejours, 1981; Seymour, 1982; Haab 1990). Oxygen is primarily stored in the lungs in diving reptiles (Burggren, 1985), but there are a few species in which oxygen is stored in the blood. Burggren and Shelton (1979) determined that approximately 88% of the oxygen requirement comes from the lung during diving in red-eared slider (*Pseudemy scripta*), as well as showing that oxygen is also stored in the lungs during the apneaic period of the terrestrial form, the Greek tortoise (*Testudo graeca*). The total amount of oxygen contained within the lungs of diving reptiles generally does not

exceeded the amount contained by their land dwelling relatives, and at times is much less (Seymour, 1982, 1989). Carbon dioxide is primarily stored in the tissues and lung during apnea (Jackson and Silverblatt, 1974; Seymour, 1982; Perry, 1983; Burggren *et al*, 1989). During voluntarily breathing, the amount of gas within the reptilian lungs is highly variable and generally relates to buoyancy (Tenny and Tenny, 1970; Jackson, 1971; Milsom and Johansen, 1975). Gas exchange is directly influenced by breath holding in reptiles because of the marked circulatory adjustments which occur during the diving response, and will be described later in this chapter.

C. *Ventilation and Gas Transfer Across the Lung During Apneic Periods*

Respiration in both terrestrial and aquatic reptiles is classified as apneic, involving a characteristic pattern of breathing which is marked by alternating periods of ventilation and breath holding. Aquatic turtles ventilate their lungs in this recurrent fashion. Breathing is, of course, naturally suppressed during a dive. This period of apnea or non-ventilation is interrupted by bursts of breathing (McCutcheon, 1943; Randall *et al.*, 1944; Burggren, 1975). The timing and frequency of ventilations are controlled by several

factors which are not completely understood, however, the basic pattern depends mainly on the degree of aquatic or terrestrial adaptation (Shelton and Boutilier, 1982). These respiratory patterns continue even when the animal is removed from the water (Johansen *et al.*, 1977). A marked increase in heart rate is observed, near the moment when breathing is resumed. This event has been termed a ventilatory tachycardia (Belkin, 1964; Millard and Johansen, 1964; Burggren, 1975). Changes in heart rate may even occur with anticipated breathing (Boyer, 1964; Burggren, 1975; Johansen *et al*, 1977; White *et al*. 1989).

A study by Crawford *et al*. (1976) reported a ventilation distribution that was homogeneous throughout the lung. Burggren *et al*. (1978), showed that the respiratory gas partial pressures varied only between 5-8 mmHg between lateral chambers of the lungs in *Trachemys scripta*. Partial pressures between investments are almost identical, and seem to indicate that there might be some type of mechanism(s) which might be mixing lung gases to prevent their stagnation and the formation of boundary layers. Changes in body posture must be taken into account because the head and limbs modulate the positions of internal organs when contracted or relaxed (Burggren *et al*., 1978). These changes in posture directly disrupt gas distribution in the lung as surrounding organs are

compressed within the coelom. The actual kinetics of gas transfer across the lung depends on the duration of the apneic period (Seymour, 1982).

Non-pulmonary gas exchange can occur through cutaneous capillaries in the dermis of the skin, and occurs in some aquatic snakes and highly aquatic turtles (Seymour, 1982, 1989; Burggren, 1985). There are a few chelonians which exhibit non-pulmonary gas exchange through the walls of the pharynx and cloaca (Seymour, 1982). However, studies on *Emys* sp. and *Pseudemys scripta* show that only low rates of non-pulmonary oxygen uptake occur, and indicate this form of gas exchange is under little physiological control and severely diffusion limited (Seymour, 1982). The keratinized scutes and scales of terrestrial reptiles are a barrier against exchanges with the environment. Terrestrial forms exhibit little or no cutaneous gas exchange due to the importance of water regulation.

D. *Relationship Between Ventilation and Lung Perfusion*

In concert with the ventilatory tachycardia (as described in section C above), there is an increase in pulmonary perfusion in *Trachemys scripta* (White and Ross, 1966; Shelton and Burggren, 1976; Johansen *et al.*, 1977).

This response is also observed in many diving mammals and birds (Johansen *et al*, 1977). When reptiles are submerged during diving, peripheral vasoconstriction decreases systemic blood flow, bradycardia develops and pulmonary blood flow is typically reduced (White and Ross, 1966; Burggren, 1975; Shelton and Burggren, 1976). Typically, terrestrial reptiles breath more quickly and more regularly than their aquatic counterparts (Seymour, 1982). These synchronous events (bursts of breathing, tachycardia, and perfusion relationship) create fluctuating gas exchange rates and marked variations in blood gas partial pressures during intermittent breathing (Burggren *et al.*, 1977).

E. *The Control of Buoyancy*

Aquatic reptiles control their specific gravity by changing lung volume. The heavy shells of turtles (Jackson, 1969) and the stomach stones of crocodilians (Cott, 1961) allow these reptiles to assume negative buoyancy even with large lung volumes. The aquatic pond turtles, (*Trachemys*) are unlike other aquatic turtles in that they possess two additional methods for controlling buoyancy, which are not present in the more highly aquatic, mud, and soft shelled turtles (Jackson, 1969). The aquatic forms (*Trachemys*) use a combination of the

cloacal bursa and the urinary bladder to modulate water volume in concert with volume changes occurring in the lungs. Jackson (1969) concluded that the cloacal bursa serves as a site for active short term water volume adjustment, while long term buoyancy adjustments are effectively regulated by the bladder. Lung volume directly affects the duration of the dive, while the specific gravity of these creatures depends on the total volume of pulmonary gas. A study by Milsom and Johansen (1975) demonstrated that the sea turtle, *Caretta caretta,* can alter its specific gravity through lung volume changes. Volume changes with the hydrostatic compression of water (in accordance with Boyle's Law). Neutral buoyancy can occur only at one particular depth. If at a point above neutral buoyancy, the animal will rise, and at any point below neutral buoyancy, it will sink. An animal simply becomes less buoyant as gas is removed from the lung (Seymour, 1982). One reptile in particular, the pelagic sea snake (*Pelamis platurus*) is able to select a particular depth for neutral buoyancy (Graham *et al.*, 1988). This snake has the ability to adjust lung volume at the onset of the dive, and concurrently rise within the water column as the lung expands. This solitary adaptation enables these reptiles to seemimgly plan the depth of its dives.

F. *Pulmonary Circulation*

Reptiles have evolved with several notable circulatory system features. The typical reptilian heart is divided into three major chambers. It consists of a ventricle (with 3 sub-chambers), two atria and a series of arterial aortic arches. There are separate right and left aortas. The common pulmonary artery arises from the cavum pulmonale, while all the systemic arteries arise from the cavum venosum. (Shelton and Burggren, 1975). There is a mixing of blood (oxygenated and deoxygenated) within the two atrial chambers, and mixing can occur during ventricular diastole and systole. In *T. scripta*, during lung ventilation approximately 65% of the cardiac output perfuses the pulmonary circulation, in comparison to 45% during a period of apnea (Shelton and Burggren, 1975). The reptilian circulatory system enables shunting of blood in a left to right fashion pulmonary bypass during ventilation and a right to left fashion during apnea. This pulmonary bypasss reduces cardiac energy expenditure (Burggren, 1985). The pulmonary bypass also maintains arterial saturation of oxygen at a low level while at the surface, and right to left shunting increases as the lung collapses during diving in reptiles (Shelton and Burggren, 1975; Seymour, 1982). Burggren (1985) also shows that the

increased perfusion of the pulmonary circuit in the left to right bypass will replenish oxygen stores and release carbon dioxide during brief ventilatory periods, exhibited in these reptiles. This adaptation is significant in that it allows for a more effective mechanism for matching lung perfusion and ventilation during breathing cycles, and a pulmonary bypass existing during apnea which directs a greater volume blood into systemic circulation (Burggren, 1985).

G. *Autorhythmic Contraction of the Lung*

Tsuchiya-Shoichi (1959) reported that the lungs of Reptilia and Amphibia exhibit a unique type of automatic rhythmic contraction, or autorhythmicity. In Japanese toads the amplitude of the pressure change reached its highest peak at between 58 Pascals (Pa) and 78 Pa at an intrapulmonary pressure of about 200 Pa. These autorhythmic contractions have also been documented by W. Burggren and A. Smits (unpublished) in Austrailian long-necked turtles (*Chelodina longicollis*). Could these fluctuations serve the function of mixing lung gases? These transient pressure fluctuations remain unstudied. This phenomenon led me to investigate the lung physiology of the red-eared slider and this recurring phenomenon.

II. The Hypothesis

A series of preliminary experiments were initiated to investigate a recurring, transient pressure fluctuation occurring during diving in the aquatic turtle, *Trachemys scripta elegans*. I hypothesize that during diving there are spontaneous lung pressure fluctuations that cause a mixing of the gases within the compartments or investments of the lung on the same side, as well as a mixing between their multicameral lungs. These lung pressure fluctuations could indicate an ability to enhance pulmonary gas exchange during a dive by stirring stagnant pockets of lung gas. Are these transient pressure fluctuations in the lungs some form of autorhythmicity? What function could they serve? The scope of this project encompassed: (1) documenting this phenomenon and its frequency, (2) assessing the mechanism(s) for these lung pressure fluctuations, and (3) establishing the physiological function of these recurring lung pressure fluctuations.

CHAPTER 2

METHODS AND MATERIALS

Experiments were performed on forty (40) individuals (0.6 - 2.4 kg body mass) of both sexes of the turtle *Trachemys scripta elegans* (the red eared-slider), a widely distributed North American species. All animals were obtained from the same animal supply house located in Nashville, Tennessee, and were subjected to a one week prophylactic treatment (one injection/day) of 0.2 ml/kg of Enroflozacin, an antibiotic, in order to help insure good health. All experiments were conducted between temperatures of 22° and 25° Celcius. Turtles were fed ad lib until experiments were begun. After the completion of physiological experiments, turtles were euthanized by a high dose of Euthan-6-ol.

This study was begun after Protocol #R701-0292-067 was approved on February 20, 1992 by the University of Nevada, Las Vegas Institutional Animal Care and Use Committee (IACUC). Funding for this project was supported by NSF Grant# IBN-930-7832 to Dr. Warren W. Burggren.

I. Histological Procedures and Techniques

The lungs of a freshly killed *T. scripta elegans* were removed by a ventral dissection, entailing removal of the plastron followed by excision of all other organ systems and associated structures. This enabled unobstructed excision of the trachea and lungs because the lungs adhere to the inner surface of the carapace and adjoining peripheral borders. Great care was taken during excision so as to not puncture any region of the lungs. The excised lungs were manually inflated with 85 ml of air by cannulating the trachea just anterior to the branching point of the primary bronchi. The inflated lungs were then fixed by complete submersion in 10% phosphate buffered formalin (Humason, 1979) for 25 hours at room temperature. One cm squares of lung tissue were excised from various regions of the fixed lung allowing for visual examination of the internal pulmonary framework. These blocks of tissue were prepared for examination with light microscopy using standard paraffin technique (Humason, 1979).

Tissue blocks were dehydrated in a graded series of ethyl alcohol, cleared in two changes of tolulene and infiltrated in paraffin at 60° Celcius. Sections were cut at 10 µm using a rotary microtome, mounted on glass

slides, hydrated, and treated with a saturated solution of picric acid in 95% ethyl alcohol to increase the effectiveness of the staining procedure, as recommended by Galigher and Kozloff (1971). Sections were stained using a modified Lillie's modification of Masson's Trichrome using Biebrich Scarlet and Fast Green FCF as counter stains (Galigher and Kozloff, 1971) and Groat's hematoxylin (Gabe, 1976) as a nuclear stain. This staining procedure was chosen because it provides for excellent differentiation of smooth muscle fibers.

II. Scute Mapping

The plastron and carapace of these animals are covered by external, keratinized plates known as scutes. The interior of the carapace is composed of dermal bone and ribs. The ribs insert into sockets located at the periphery of the dorsal carapace, where they form a gomphosis. The scutes of the plastron and carapace can be used to map the locations of underlying structures (organs, muscles, or blood vessels). Scute mapping is highly useful because it permits accurate placement of cannulae and electrodes, as well as allowing easy repetition of experiments.

III. Experimental Conditions

All experiments were conducted between the hours of 0700 A.M. and 0200 A.M. All dives were voluntary and animals were unrestrained. Animals were kept under a standard 12 hour day and 12 hour night cycle. In order to minimize any additional stress due to visual disturbances, tanks (rectangular 5 gallon, 8.5 X 16.5 inch) were completely enclosed with opaque material and covered with a styrofoam lid. Turtles almost always rested on the bottom of the tank. When the need arose to breath, turtles simply extended their necks until their nostrils were above the surface of water. Turtles only swam when the water level in the tank exceeded the reach of the neck, forcing them to raise themselves in the water column high enough to reach the air. When they were through breathing, turtles returned to the bottom of the tank.

IV. Surgical Procedures

Turtles were intubated and anesthetized with a mixture of carbon dioxide, Halothane, and air (0.3%, 5.0%, and 94.7% respectively), administered with a Foregger Fluomatic anesthetic dispenser. Sites of the plastron and carapace were coelomic invasion occurred were treated with Betadine, a broad-spectrum antiseptic, before surgeries were performed. Animals were artificially ventilated with

a Harvard Apparatus Respirator (9 ml at 8 strokes per minute) for no more than an hour after surgery, and then placed in a recovery tank for twenty-four hours before any experiments were initiated. This allowed for a complete recovery.

A. Cannulations

For lung cannulations, a small region (0.9 cm. x 0.9 cm) of a desired costal scute and the underlying bone was exised using a Model 260 Dremel moto-tool. Underlying connective tissue was carefully cut away. Once an area had been identified for cannulation, a small incision was made in the skeletal muscle tissue overlying that region. A lung investment was selected, and then a small portion of that investment extracted through the hole with hemostats. Care was taken in selecting a region which was devoid of superficial blood vessels, in order to minimize trauma to the lung. A small incision in the lung wall was made, into which a 15 cm long polyethylene (PE)-205 cannulae was inserted. This tubing was modified with a flared region approximately 0.6 cm away from the end of the cannulae, followed with 3-5 holes bevelled near the actual tip. Care was taken to insure that the tip of the cannulae came to rest within the central lumen of the investment. The incision site was then sutured closed around the cannulae. The cannulated lung investment was

then allowed to return to its normal resting position. An exit port for the cannulae was drilled into the excised region of scute and bone, and then replaced. All holes and excised regions were sealed with wax, and then covered with epoxy.

B. *Measurement of Abdominal Cavity Pressures*

In order to assess how forces act to alter internal lung pressure, a second fluid-filled cannulae (PE-205) with a modified rubber tip attached to the end of the cannulae was positioned in the coelomic cavity in four turtles. The modified rubber tip was made by taking a surgical glove and cutting off the distal (1.25 cm) portion of a finger tip. The cannulae and finger tip were submerged in saline and the rubber tip fastened onto the end of the saline filled cannulae with Ethicon surgical thread. The rubber tip at the end of the cannulae was then slightly inflated and calibrated. This second cannulae would allow comparison of coelomic cavity pressures with those of the lung. An exit port was also drilled for this tubing in the excised region of scute and bone (see ***Cannulations*** section above).

C. *Atropine Delivery and Electrode Placement*

In ten experiments, turtles were turned onto their dorsal surface (ventral side up), following lung cannulations. Three one mm diameter holes were drilled one cm. apart into the plastron directly above the heart. The most posterior hole was fitted with a PE - 50 cannulae whose tip was advanced (1.0-1.5 cm) into the coelom, while the other two anterior holes were fitted with insulated copper wire. The wire tip was exposed and inserted 0.5-0.75 mm into the hole. Atropine sulfate was delivered intramuscularly (IM), intraperitoneally (IP), and later attempted intrapericardially. Preliminary testing determined that IP was the most efficient manner because it allowed the delivery of atropine without inducing stress due to either handling of the animal or trauma of an IM injection. The PE - 50 tubing served for IP deliver of atropine sulfate, a muscarinic blocking agent. At high dosages atropine inactivates smooth muscle (Weiner, 1985). Smooth muscle may play a role in creating recurring lung pressure fluctations during diving. Atropine blocks the vagal component of the nervous system causing heart rate to rise above 40 beats/min, so electrocardiograms were used to monitor the effective dosages and delivery of the atropine into the circulatory system.

V. Pressure and ECG Measurements

Lung and abdominal pressures were measured through the polyethylene cannulae with Narco P-1000B pressure transducers whose outputs were fed into a Narco Systems (four channel) Physiograph. Electrocardiograms were produced by feeding the insulated copper leads (two) into a high-gain coupler and channel amplifier, also connected to the Narco Physiograph. The majority of experiments were conducted with turtles in a tank containing ten cm of water, but LPTs were also examined at water depths of fifteen and twenty cm. Once animals had recovered from surgery, they were left unrestrained in a tank for an 8-24 hour control period during which lung pressures during voluntary dives were recorded. At the end of the control period atropine sulfate was administered (see **Atropine Delivery and Electrode Placement** section above) and the same animal was again allowed to voluntarily dive (unrestrained in any way) until the effects of atropine had worn off. A large rise in heart rate is an indicator that Atropine has entered the circulatory system, and could be accomplished by monitoring heart rate via an electocardiogram.

VI. Measurement of Helium Redistribution Between Lungs and Within the Same Lung

Two major experiments were conducted to evaluate the hypothesis that during diving there are recurring, transient lung pressure fluctuations that cause a mixing of gases within the compartments or investments of the lungs on the same side, as well as a mixing between lungs. The movement of helium (He) between lungs and the passage between the most posterior and anterior investments of the same lung were studied. Helium, an inert gas, was chosen for this experiment because it has a solubility of less than 0.01 percent in the lungs, plasma, blood, and in muscle tissue (Weathersby and Homer, 1980). Once two different sites of a lung(s) were cannulated, lung gases and LPTs were monitored through the PE - 205 tubing. One (primary) site was used specifically to monitor for LPTs and to deliver a bolus of pure helium, while the other (secondary) site was used for direct sampling of lung gases (Figs. 1B and 1C). Lung gases were drawn directly into a VG 200D Quadropole Mass Spectrometer for analysis. The Mass Spectometer was calibrated before each experiment using a Whostoff Gas Mixing Pump (Bochum, Germany).

The protocol consisted of injection of a known quantity of helium into one investment, followed by direct sampling (by the Mass Specrometer) for four seconds from

the secondary site. Preliminary testing determined that a four (4) ml bolus should be used for tests conducted to determine the movement between lungs, while a 0.5 ml bolus should be used to examine the movement of He between investments of a single lung. The rate of the appearance of He at the secondary site was then recorded. All measurements were made during a single episode of apnea uninterrupted by breathing. In testing the movement of He between two lungs, samples were taken at one, three, and five minutes after the inital injection of He, and then once every 2 minutes after He was detected. In testing the movement of He in the same lung (posterior to anterior investment), sampling occurred once every minute for the first seven minutes and then every other minute afterwards. The highest He concentration recorded by the Mass Spectrometer for each sample interval was plotted on a graph (X coordinate=elapsed time, Y coordinate=highest helium point for that interval). A linear regression was then generated through all the helium points up to the peak point before the first breath (Fig. 8). A comparison was made by evaluating the difference between the averages of slopes in trials exhibiting lung pressure transients against ones which were not.

VII. Statistical Comparison of Data

Non-paired t-tests were used to determine significance between groups of control and atropine experiments conducted at the three different water depths. Paired t-tests were used to determine significance between the averages of slopes generated in experiments testing the movement of helium between and within the same lung. This was possible because each animal provided a control (dives experiencing LPTs) and an experimental value (dives absent of LPTs). When parametric tests were violated, analysis were performed using the Mann-Whitney Rank Sum Test, a nonparametric treatment. A Kruskal-Wallis One Way Analysis of Variance on Ranks (ANOVA) was used to determine significance between different dive length intervals and the frequency of LPTs per dive. All statistical treatments were performed by a Gateway 2000 4DX2-66V computer using SigmaStat Version 1.0 from Jandel Scientific (San Rafael, California). R and R^2 values were generated for the linear regressions by computer in order to show fitness of those regression lines in helium passage experiments. All graphs were produced using SigmaPlot Version 5.0 from Jandel Scientific (San Rafael, California).

CHAPTER 3

RESULTS

I. Histological Sections and Interpretations

Representative drawings of sagital sections through the left lung of *T. scripta elegans* excised for histological preparation are represented in Figure 1A. Lung cannuales were placed in specific locations and would be used for gas sampling, pressure monitoring, and for injections of helium (Fig. 1B and 1C).

Perry (1983) showed that this type of lung is subdivided into various bilaterally symmetrical chambers, an arrangement he termed multicameral. Although bilaterally symmetrical, adjacent chambers of a single lung were not identical in size or shape. Burggren *et al.*, (1978) described a first generation bronchus which immediately divided into long posterior branches and shorter anterior branches. The present observations support this description. The lungs are voluminous, with each chamber containing a central lumen free of any tissue. The most anterior investments are highly vascularized and predominantly large blood vessels (1 mm

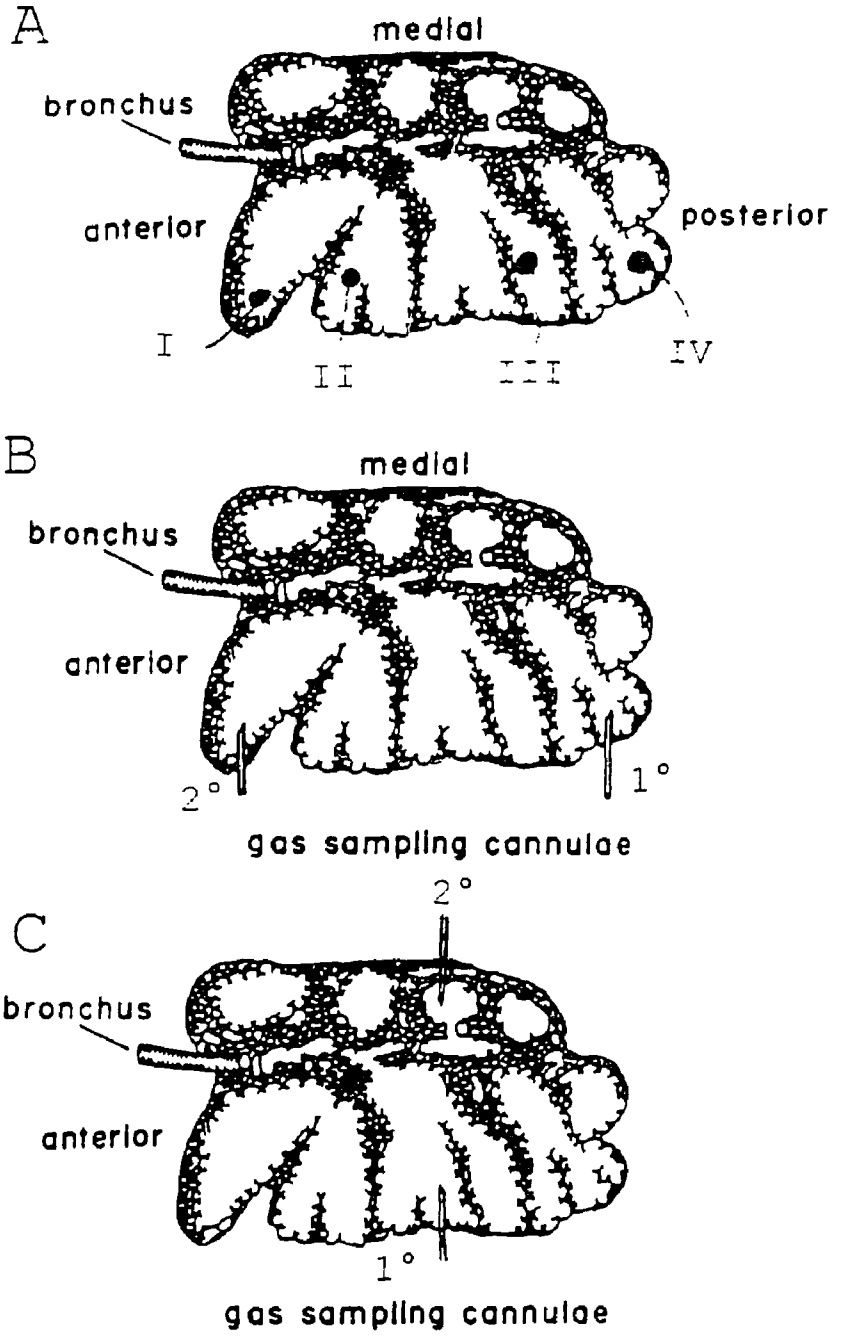


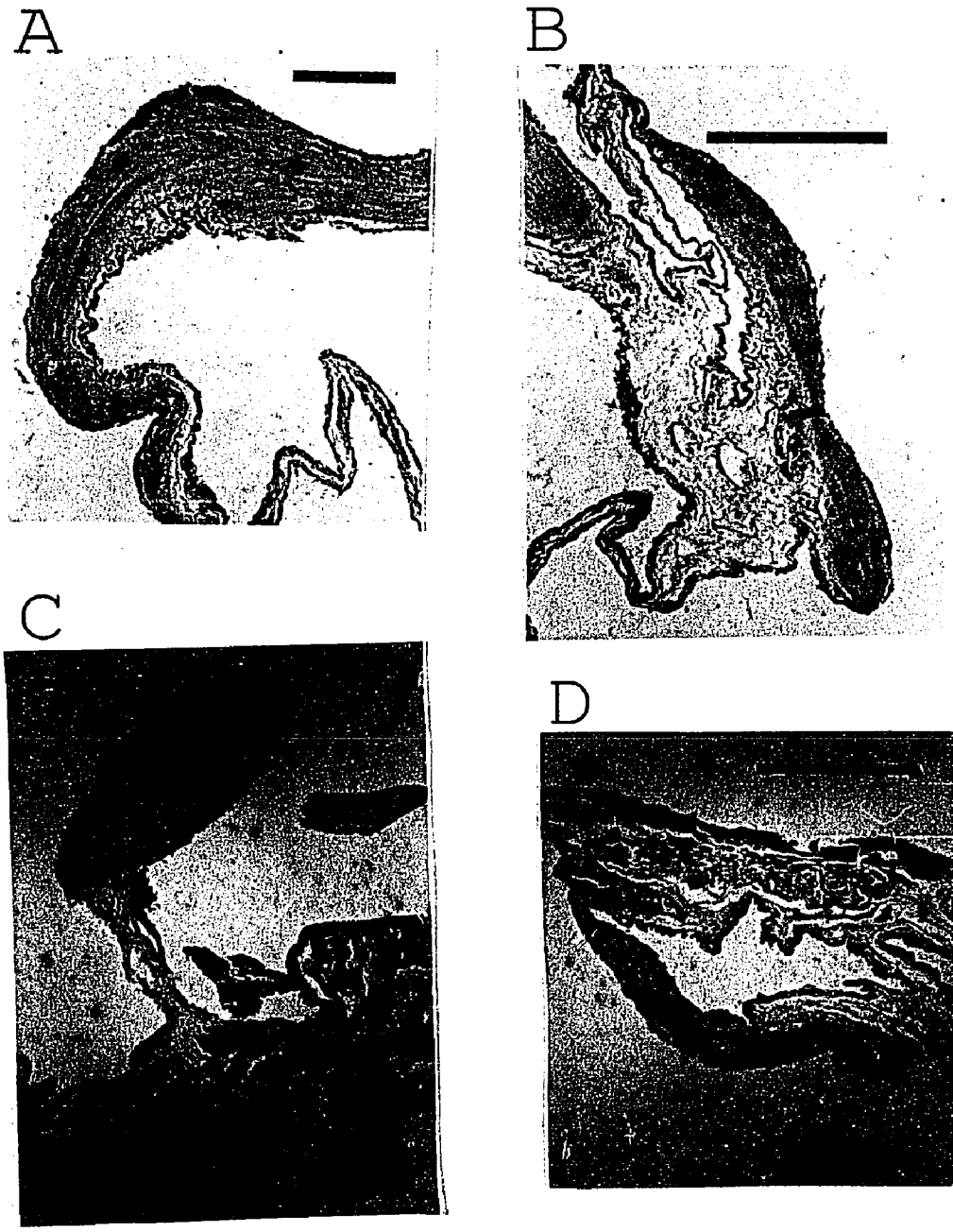
Figure 1A. Sagital section of the lung of *T. scripta elegans*. Figures 1B and 1C show locations of cannulae placement used for pressure measurements, gas sampling, and helium injections (modified from Burggren *et al.*, 1978). 1° and 2° notation represent gas sampling sites (see section VI of Methods and Materials). Roman numerals represent area removed for histological preparation.

diameter) encompass the outer lining of these investments as well. Internally, each investment contains a honeycomb-like structure formed from trabeculae which are composed of smooth muscle and elastic connective tissue. This polygonal network has been termed edicular, Dunker (1981).

Smooth muscle lies in and near the superficial surface of the lung (Fig. 2A-D). The specific orientation of this smooth muscle has not been clearly determined. The lung wall is primarily composed of elastic connective tissue, collagen fibers and some smooth muscle (Fig. 2B and 2D; regions IV and II respectively). Each chamber is composed of a polygonal network comprised of smooth muscle bands and elastic connective tissue called trabeculae (Fig. 2A and 2C; region I and IV respectively). Clearly, there are broad quantites of smooth muscle in the construction of lung (Figs. 2A-D).

II. Lung and Intraabdominal Cavity Pressure Comparisons

In this experiment the pressures within the lung and coelomic cavities were monitored in an unrestrained animal during a series of voluntary dives (Fig. 3). A tachycardia is generated during breathing or in its anticipation and can be monitored by electrocardiagram



Figures 2 A-D. Histological preparations of the lung wall and trabeculae of the turtle, *Trachemys scripta elegans*. Bar lengths in all figures represent 0.1 mm.

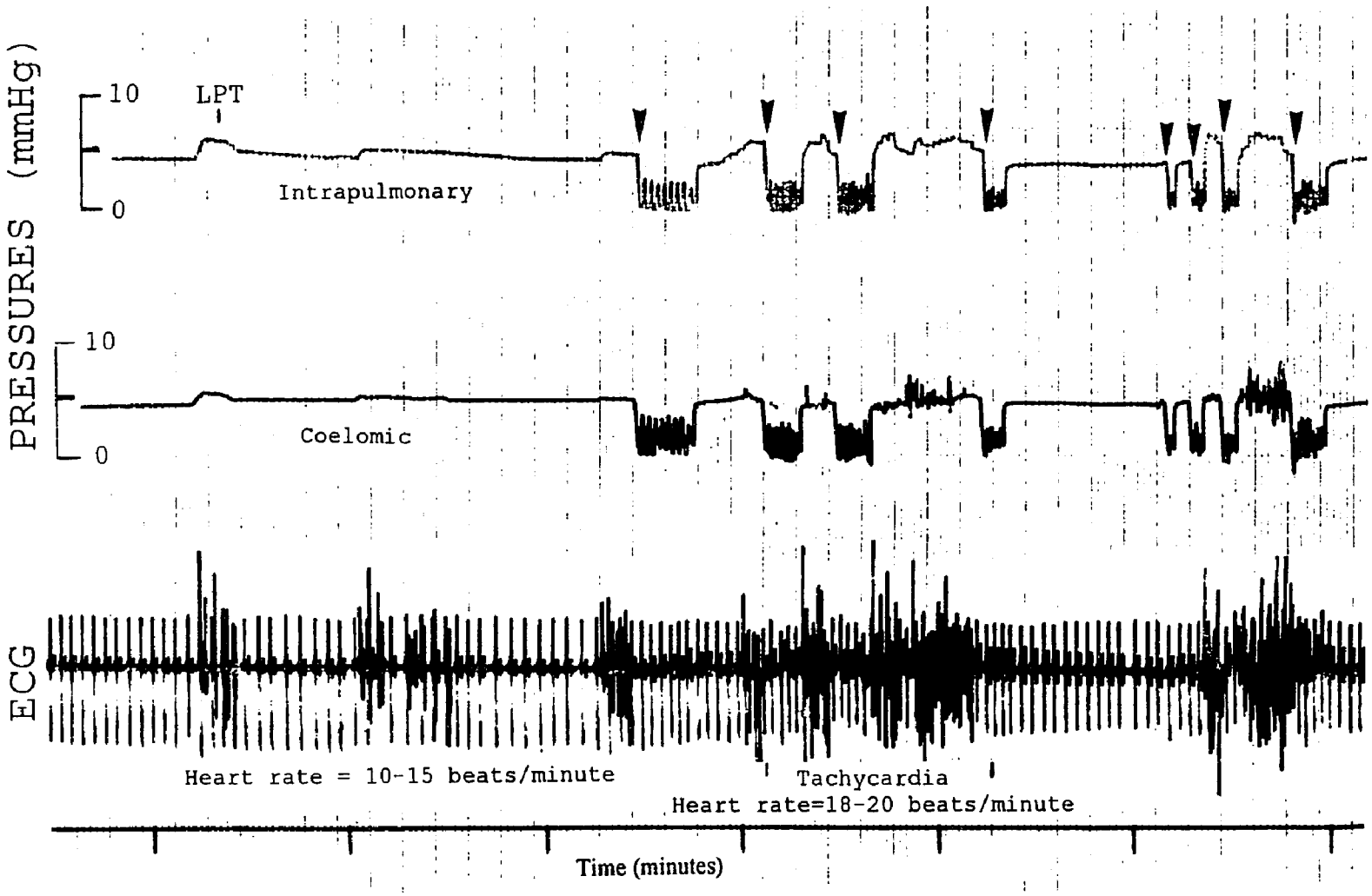


Figure 3. Representative patterns of intrapulmonary (upper trace) and coelomic pressures accompanied by an electrocardiogram during several periods of apnea and breathing sequences generated by an unresrained *T. scripta elegans*. Solid black arrows indicate the onset of breathing. LPT = Lung Pressure Transient. Time marker in minutes.

(Fig. 3). This figure also illustrates one transient pressure fluctuation occurring during the dive; henceforth termed a "Lung Pressure Transient" (LPT). LPTs during diving were defined as a spontaneous increase in lung pressure of at least 0.3 mmHg above that of the intrapulmonary pressure exhibited at that particular depth. The same variables (ECG, intrapulmonry and coelomic cavity pressures) were monitored as in Figure 3 with the exception that this animal was injected with a high dose of atropine sulfate (3.0 mg/kg) that served to inactivate smooth muscle contractions. The electrocardiogram depicts the elevated heart rate that occurs as a result of the blockage of the vagal component of the nervous system (Fig. 4). Identical pressure relationships occur between both the coelomic and pulmonary cavities during dives and while breathing (Figs. 3 and 4).

III. Relationship Between Dive Depths and Occurrence of LPTs

The mean hourly frequency of LPTs in control animals and in atropinized animals at three different tank depths (10, 15 and 20 cm) were recorded (Fig. 5). For this experiment, the total number of LPTs occurring for each depth was multiplied by 60 minutes and then divided by the

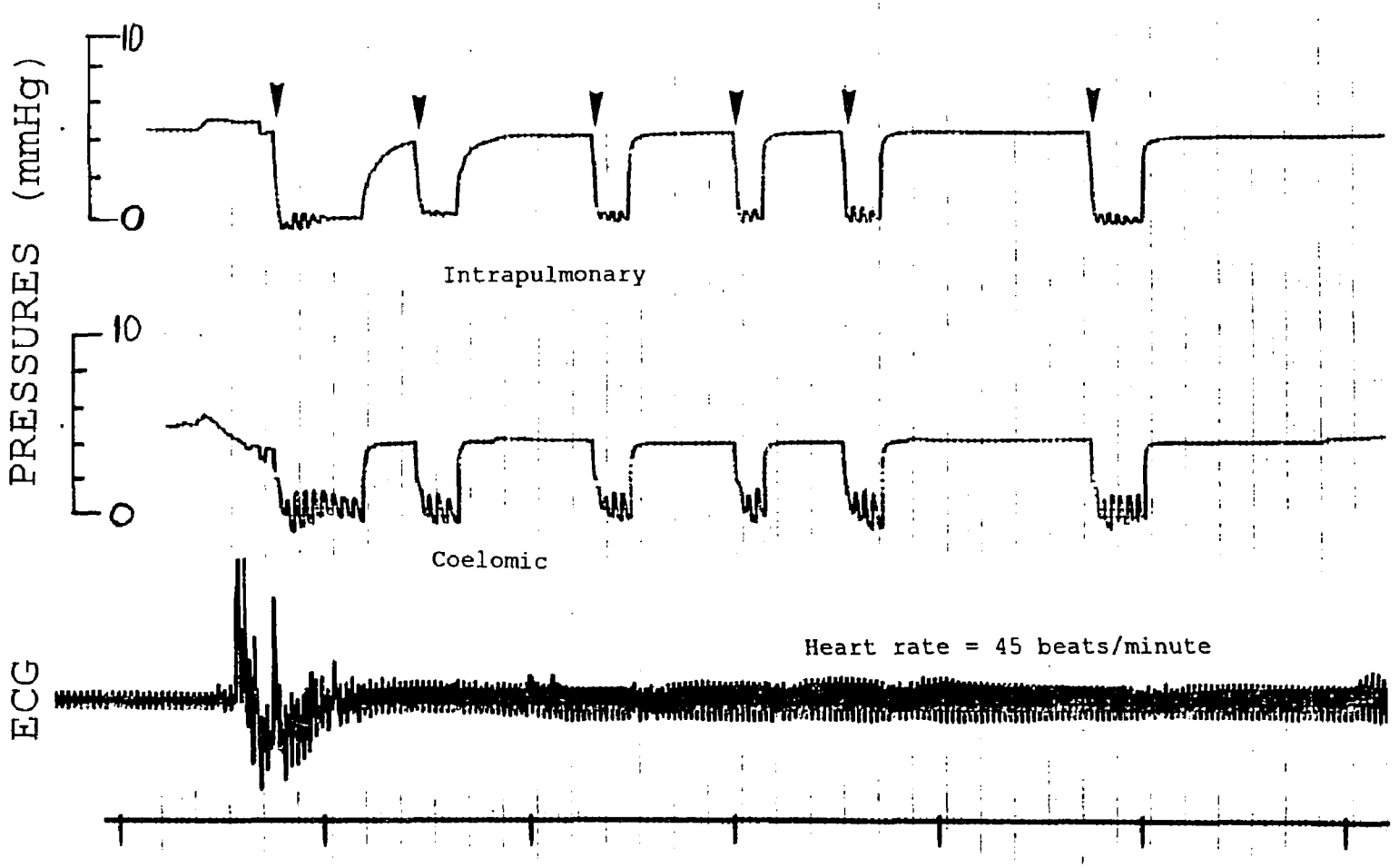


Figure 4. Representative patterns of intrapulmonary and coelomic pressures accompanied by an electrocardiogram during several periods of apnea and breathing sequences generated in an unrestrained, atropinized *T. scripta elegans*. Solid black arrows indicate the onset of breathing. Time marker in minutes.

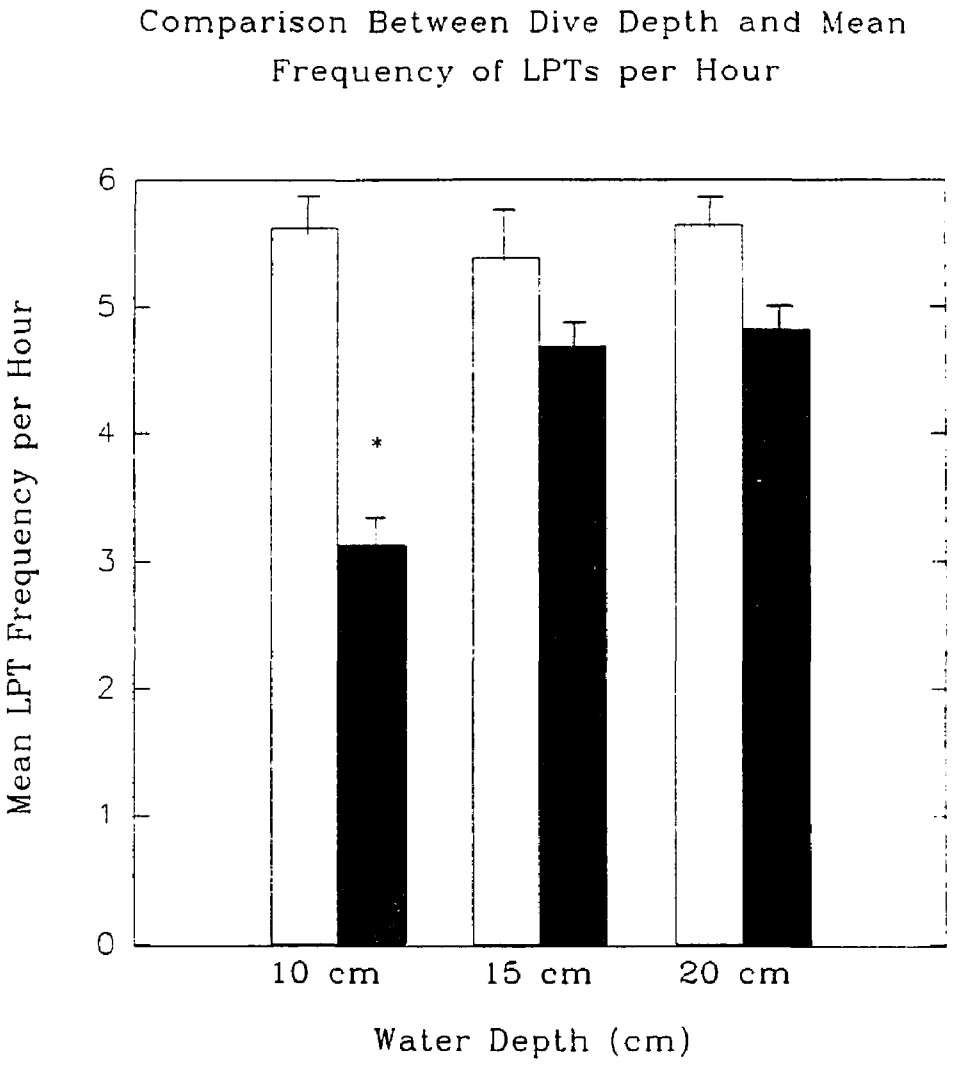


Figure 5. Comparison between dive depth and mean frequency of LPTs per hour. Open bars = control turtles, shaded bars = atropinized turtles. * = significant difference between atropine and controls at $P<0.05$ level. Standard error bars are shown.

total minutes of apnea for that dive interval [LPTs / (total apnea/60) = LPTs / hr]. For experiments with a water depth of 10 cm, twelve turtles were used as controls and consequently atropinized, respectively recording 8,090 and 2,690 minutes of apnea. For experiments with a water depth of 15 cm, seven turtles were used as controls and then atropinized, respectively recording 903 and 719 minutes of apnea. For experiments with a water depth of 20 cm, three turtles were used producing 447 and 411 minutes of apnea respectively. In control dives LPTs are produced with approximately the same frequency regardless of depth, and are still generated even in atropinized animals (Fig. 5). However, their frequency is reduced at the 10 cm level, but not at the 15 or 20 cm levels (Fig. 5). Student's t-tests were performed comparing only controls and atropinized animals at that same tank depth. At the 10 cm water depth, mean values of LPTs were significantly different between control and atropinized animals ($P<0.05$, t-test), but at the 15 cm water depth there was statistically no difference between the two groups ($P>0.05$, t-test). The small number of turtles (3) analyzed at the 20 cm water depth negates the validity of statistical analysis.

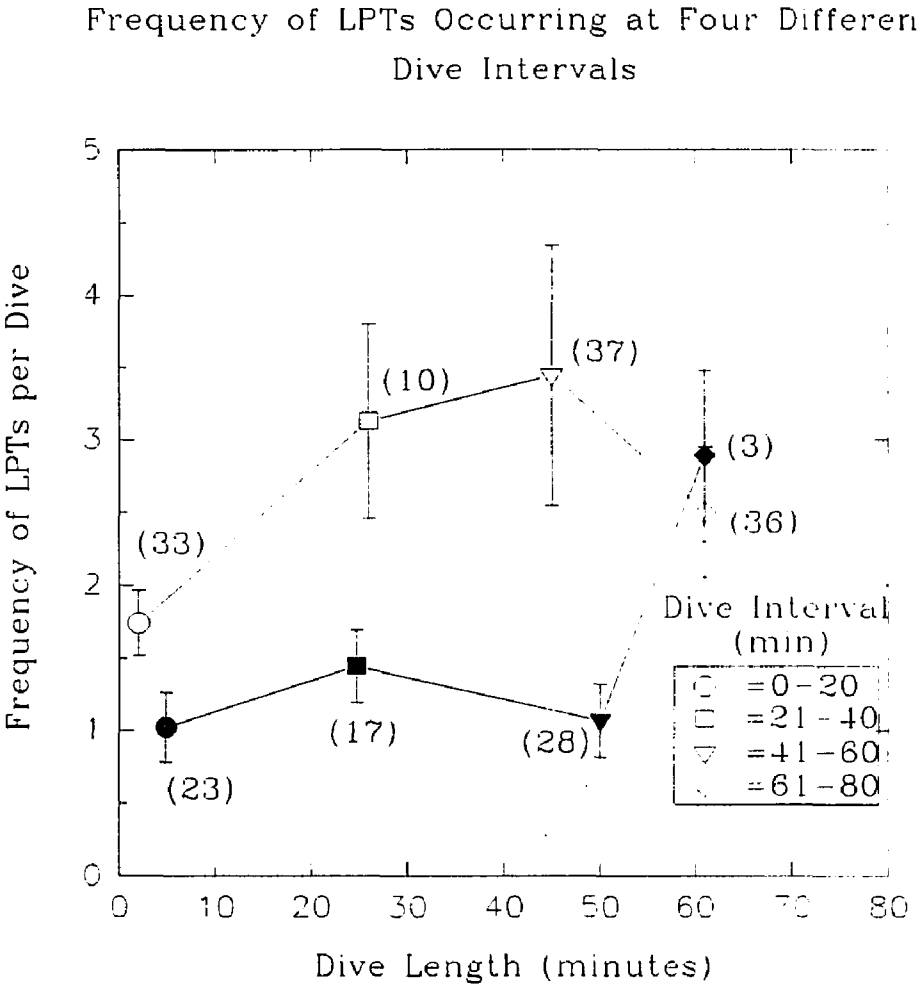


Figure 6. Frequency of LPTs occurring at four different dive intervals. Open symbols=controls, shaded symbols - atropinized animals. Numbers in parenthesis are total number of dives measured for that interval. Standard error bars are shown.

IV. Comparison Between Dive Length and LPT Frequency

This experiment monitored the number of LPTs generated per dive as a function of dive length (Fig. 6). Dives were separated into four groups based on dive length. The four dive intervals consist of dives lasting between 0 and 20, 21 to 40, 41 to 60, and 61 to 80 minutes in duration. Data from both control and atropine experiments were separately subjected to an analysis of variance (ANOVA) which indicated that there was a significant ($P<0.01$) relationship between the median points in LPT frequency in each dive interval from both control and atropinized groups. A multiple comparisons test revealed that there was no significant difference between any of the control intervals or between any of the atropine intervals. A reduced number of LPTs in atropinized turtles were recorded in each dive interval with the exception of the 61-80 minute interval in which a greater number of LPTs were monitored as compared to the other atropinized intervals (Fig. 6).

The same data was replotted to show the frequency of LPTs per hour as a function of the four dive intervals (Fig. 7). The average number of LPTs decreases with increasing dive length in all control experiments, and the trend is consistent even within the atropinized animals with the exception of dives longer than 61 minutes (Fig.

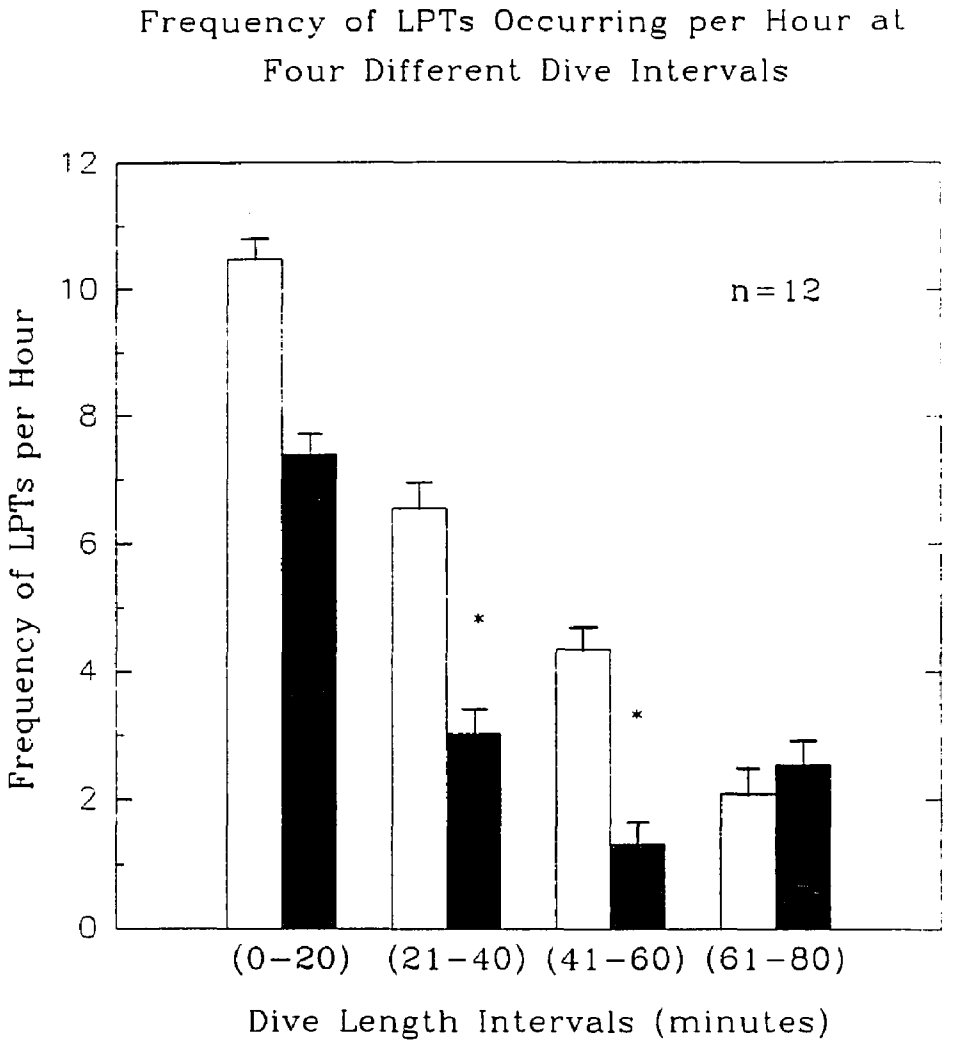


Figure 7. Frequency of LPTs at four different dive intervals. Open bars=control turtles, shaded bars= atropinized turtles. * = significant difference between control and atropine trials at $P<0.05$ level.

7). In dive intervals 0 to 20 minutes and 61-80 minutes, there was no significant difference ($P>0.05$, t-test) between control and atropinized turtles. In dives lasting 21 to 40 and 41 to 60 minutes the two groups were significantly different ($P<0.05$, t-test).

V. Helium Distribution in the Same Lung

Each animal served as its own control for these experiments. Lung pressure was monitored before and after an injection of helium to determine whether LPTs were present or absent in a particular experiment. Helium injections were made in dives with and without spontaneously occurring LPTs. Turtles that did not have at least one successful helium injection during a dive with an LPT and during a dive without an LPT were excluded from the experiment. Statistical significance was based upon the slope of the line created by a linear regression plotted through all of the helium points up to the peak point (of helium) before the first breath was taken, for that trial. A typical graph for an individual experiment recording the rate of helium appearance at the secondary site (Fig. 1B) based on elapsed time after helium was injected at the primary site, is presented in Figure 8. The rate of helium appearance in the most anterior investment (2° site) of the same lung following injection

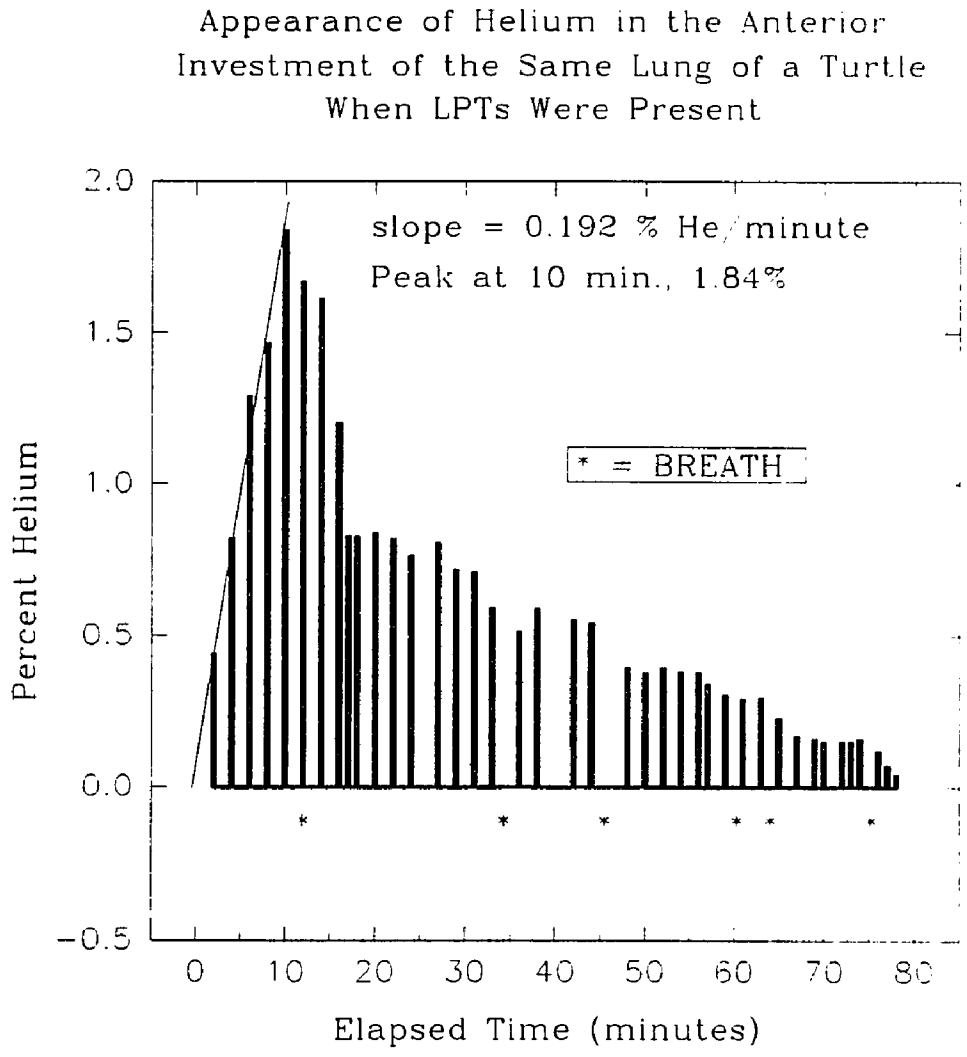


Figure 8. Appearance of helium in the anterior investment of the same lung of Turtle #35. Trial #2 when LPTs were present.

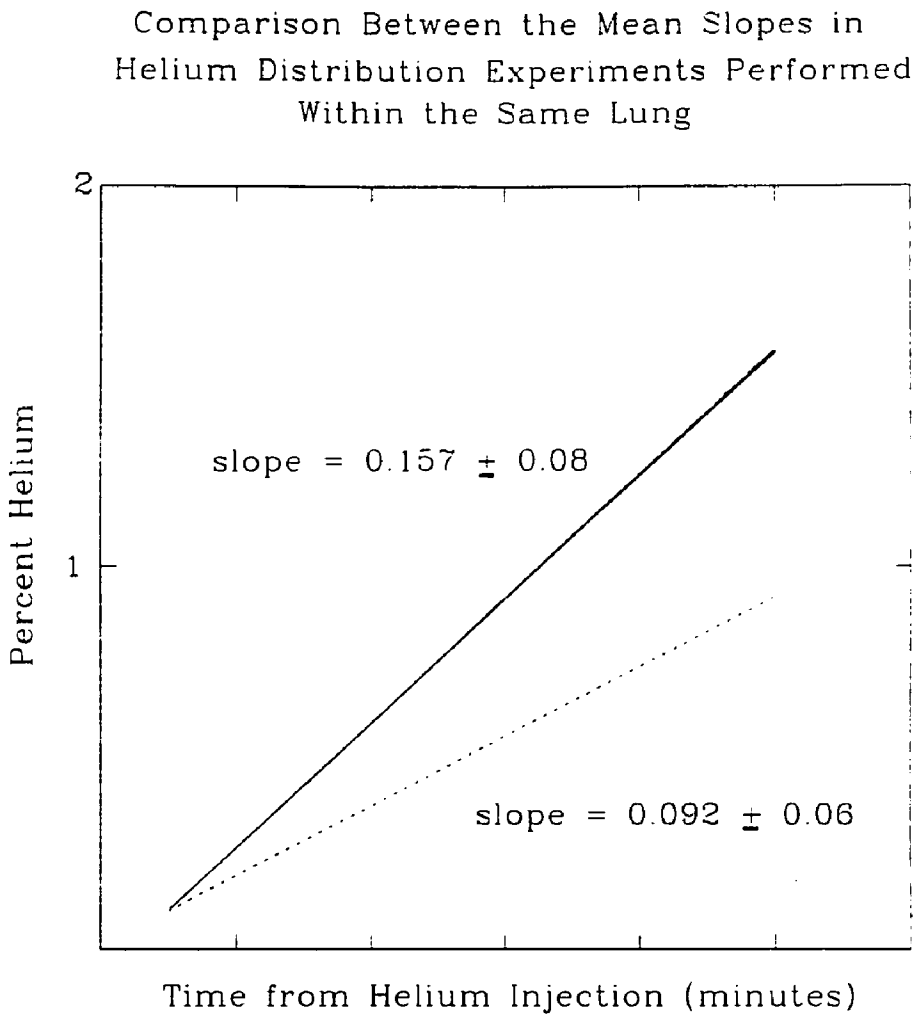


Figure 9. Comparison between mean slopes in distribution experiments in which LPTs were present and absent. Solid line represents experiments in which LPTs were present. Dashed line represents experiments in which LPTs were absent. Mean slopes and standard deviations are shown. Slopes in percent helium change/minute.

of a 0.5 ml helium bolus in the most posterior investment (1° site) occurred at a faster rate based on the mean slopes generated between animals exhibiting LPTs versus those in which LPTs were absent (Fig. 9). (A summary of all the individual trials can be found in Table 3 located in Appendix Two). Preliminary tests showed that there was no significant change in the rates of helium appearance whether measuring from the anterior to the posterior or posterior to anterior investments. Based on these preliminary findings, all experiments were conducted with helium injected into the posterior investment and sampling occurring anteriorly.

A comparison between the rates of helium appearance in animals exhibiting LPTs, against one which were not in single lung distribution experiments, were compared. The averages of slopes generated between trials when LPTs were present and those in which LPTs were not, are presented in Table 1. Four of the five turtles showed that distribution of helium occurred at least 1.3 fold faster when LPTs were present. This included one trial which expressed a greater than 6 fold increase over trials where LPTs were absent. Based on the difference of the means between the two groups, animals exhibiting LPTs mixed gases between the investments of the same side of their lungs approximately 1.5 times faster than those which did

Table 1. Average of Slopes (Percent Change in Helium/minute) Generated by a Regression Plotted Through Helium Points Recorded Before the First Breath Taken by a Turtle in an Individual Trial.

Helium Distribution in the Same Lung

TURTLE#	LPTs present	LPTs absent
35	0.134	0.020
36	0.063	0.074
37	0.137	0.070
39	0.286	0.176
41	0.164	0.120
Mean ± Std. Dev.	0.157 ± 0.08	0.092 ± 0.06

not. The difference in slopes between these two groups were significant ($P<0.05$, paired t-test).

VI. Helium Redistribution Between Lungs

Graphs were produced on the same premises as stated in the above experiment, with the exception that a four (4.0) ml helium bolus was injected into the primary site followed by sampling from the opposite lung at the secondary site (Fig. 1C). Regressions and statistical significance followed the same techniques and procedures as in Section V. The rate of helium appearance at the secondary site occurred at approximately twice as rapidly based on the means of the slopes generated between animals exhibiting LPTs versus those in which LPTs were absent (Fig. 10). (A summary of all the individual trials can be located in Appendix Two.)

The averages of slopes generated between trials when LPTs were present and those in which there were not, are presented in Table 2. In three of the five turtles, the rate of He distribution from one lung to the other was at least three times greater in turtles exhibiting LPTs versus ones which were not. This included one turtle which showed a distribution approximately 5.5 fold faster when LPTs were present. Based on the difference of the means calculated between the two groups, animals

exhibiting LPTs mixed gases between the two sides of their lungs approximately two times faster than those which were not exhibiting LPTs (Table 2). The difference in slopes between the two groups were significant ($P<0.05$, paired t-test).

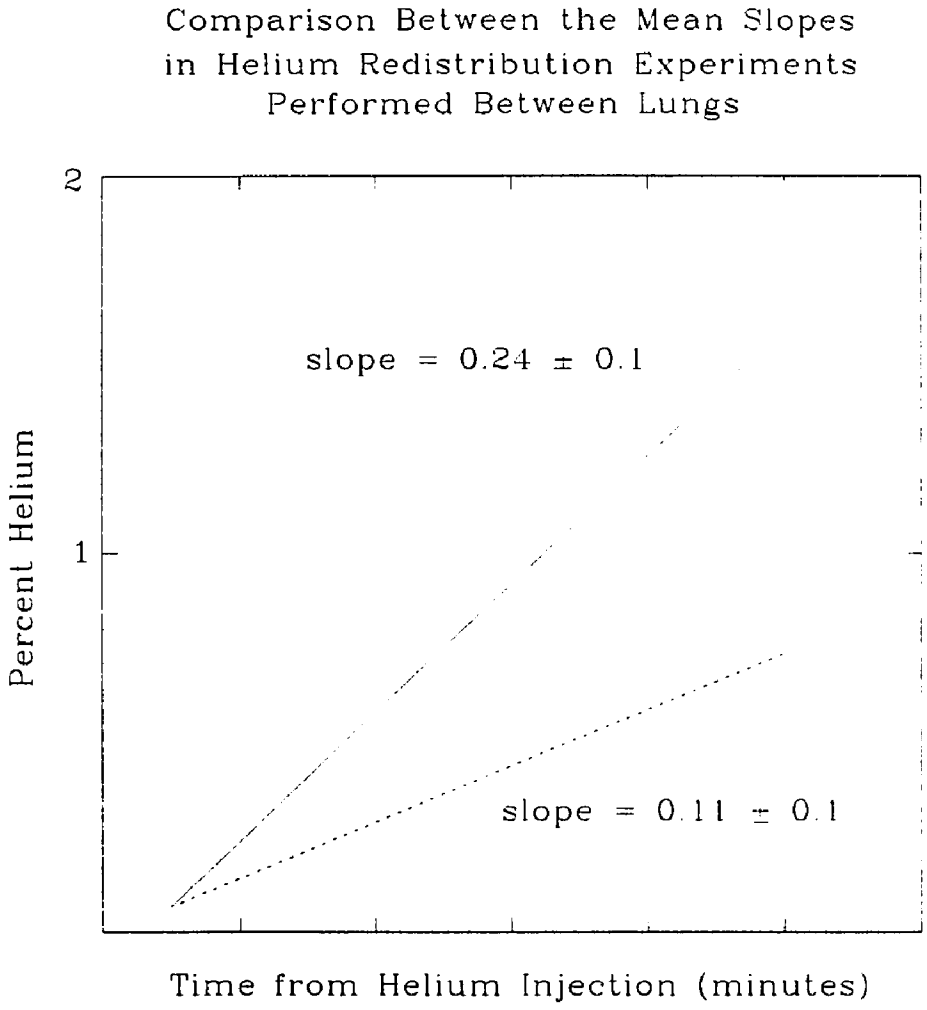


Figure 10. Comparison Between Mean Slopes in Redistribution Experiments in Which LPTs Were Present and Absent. Solid line represents experiments in which LPTs were present. Dashed line represents experiments in which LPTs were absent. Mean slopes and standard deviations are shown. Slopes in percent helium change/ minute.

Table 2. Average of Slopes (Percent Change in Helium/minute) Generated by a Regression Plotted Through Helium Points Recorded Before the First Breath Taken by a Turtle in an Individual Trial.

Helium Redistribution Between Lungs

TURTLE#	LPTs present	LPTs absent
26	0.153	0.05
27	0.279	0.091
29	0.061	0.045
30	0.332	0.057
31	0.394	0.308
Mean ± Std. Dev.	0.24 ± 0.1	0.11 ± 0.1

CHAPTER 4

DISCUSSION

In chelonian reptiles, lung ventilation is a highly variable, intermittent process. Periods of apnea may last as long as several hours or as short as a few minutes or even a few seconds (McCutcheon, 1943; Shelton and Boutilier, 1982; Burggren *et al.*, 1988; Milsom, 1988). Diving reptiles must constantly adjust cardiovascular perfusion to match effectively lung perfusion and lung ventilation (Burggren, 1975; Shelton and Burggren 1976; Burggren *et al*, 1977) to achieve economical and efficient gas exchange. Many different factors can disrupt this relationship by altering the pattern of pulmonary ventilation and perfusion. Temperature (Lucey and House, 1977; Glass *et al.*, 1985), postural positioning (Burggren *et al.*, 1978) and carbon dioxide concentrations (Funk and Milsom, 1987), are such factors. Experimentally induced changes in lung volume and pressure can mimic the normal response occurring in lung perfusion and heart rate prior to breathing (Johansen *et al.*, 1977). The evolution and

adaptations of diving reptiles has produced mechanisms with tight cohesion between cardiovascular and respiratory systems.

The lung structure in *T. scripta elegans* is multicameral (Perry, 1978) and bilaterally symmetrical, however, the adjacent chambers on either side are not identical in size or shape. Burggren *et al.*, (1978) conducted an experiment which showed the distribution of respiratory gas partial pressures along the longitudinal axis of the lungs of *Trachemys scripta*, *Testudo graeca*, and *Testudo hermanni*. The gas partial pressures varied by at most a few mmHg between all of the lateral chambers, suggesting that ventilation and perfusion are precisely matched in the lung. The morphology of the lungs in semi-aquatic turtles reveals chambers which lack both an extensive bronchiole arrangement and broad alveolization as well as having adjacent chambers which are unevenly structured with regard to gas exchanging surfaces. This morphology indicates that stagnation of gases could occur between the chambers since each chamber possesses an open lumen. Is it possible that diving reptiles possess some mechanism to internally stir gas within the chambers of the lungs in order to keep partial pressure uniformity between chambers, and hence enhance gas exchange? It would seem appropriate that since the central free lumen

is devoid of respiratory gas exchange surfaces and that the lungs of this species lack uniform alveolization, some mechanism must be present to stir lung gases. In terms of the pulmonary ventilatory distribution, the lungs are equally ventilated in terms of alveolar gas even though tidal volumes constitute approximately ten percent of total lung volume (Crawford *et al.*, 1976; Burggren *et al.*, 1978). A review explaining the aspect of boundary layers which form due to the morphology of this type of lung, and how this might be physiologically significant is warranted. It has been suggested that their exists a diffusion boundary layer consisting of stagnant layers of fluid or liquid that form immediately adjacent to gas exchange surfaces. This layer exists whether in a gas or liquid media, and occurs even if the fluid is well mixed (Barry and Diamond 1984; Feder and Pinder, 1988). In a review by Feder and Pinder (1988), these researchers determined that around stationary objects immersed in moving fluids (air or water) is a region of fluid, termed the fluid dynamic boundary layer. This gradient existed between a substance's concentration at the surface-fluid interface and its concentration in the free-stream of moving fluid; the region in which the concentration differs from the free-stream concentration is the diffusion boundary layer.

I. Mechanism for Generating LPTs

The lungs of amphibians and reptiles often exhibit a type of automatism or autorhythmic contraction (Tsuchiya, 1959). Isolated lung experiments revealed that the automatism developed in the first 15 to 60 minutes and lasted for several hours. The most active and persistent automatism was detected in the apex regions of the lung. The study also suggests that temperature and seasonality may have some significant effect. This autorhythmic phenomenon occurring in the lungs is also documented in the pressure recordings of Burggren *et al.* (1978) and W. Burggren and A. Smits (unpublished). The only two physiological mechanisms that could create this autorhythmic contraction in the lung would seem to be contraction of smooth or skeletal muscle. There are broad quantities of smooth muscle in the lining of the lungs and in the internal construction of the trabeculae (Figs. 2A-2D). The contraction of smooth muscle, by changing lung volume, could assist in correcting buoyancy problems, and probably assist in lung contractions, similar to the function to the smooth muscle found in the trabeculae (Perry, 1983).

Skeletal muscle, however, could also produce lung pressure and volume changes. The evolution of the rigid shell and the skeletal adaptations in turtles has received substantial attention by vertebrate zoologists because of the implications they create for the respiratory system. The respiratory maneuvers in other organisms is missing because of the uncompromising shell in turtles, which makes free movements of the body wall impossible. Hence, the mechanism of gas exchange in turtles is entirely different from those of other reptiles. The lungs of a turtle are basically enclosed in solid "box" which has five protruding structures (four limbs and the head) which can either be extended outward, or retracted inside the box. When a limb or the head is either partially or fully retracted into the shell, it actively displaces the other organs inside the shell. This action in turn causes the pressure within the coelom to increase. This pressure is then exerted onto the lung. Numerous studies have documented non-locomotory limb movements in stationary animals (McCutcheon, 1943; Gans and Shah 1955; Perry, 1983), and make the assumption that these actions are deliberate and serve some purpose. The alternating contractions of specific skeletal muscle groups were shown to be the primary mechanism responsible for creating lung volume changes in turtles (McCutcheon 1943; George and

Shah, 1955; Gans and Hughes, 1976; Perry, 1978). All prior work leads to the assumption that reduced contractions of these same muscle groups could also serve to create LPTs as well. The present study indicates that LPTs are generated during times of limb movement(s). However, LPTs also occurred when animals sit motionless. Identical pressure relationships (Figs. 3 and 4) occur between the abdominal and pulmonary cavities during dives and while breathing. Close examination seem to reveal that at the onset of breathing, pressure changes occur simultaneously in the coelom and in the lung. Figure 3 illustrates that the mechanism(s) responsible for creating LPTs is not mediated at the level of the lung, but rather by some mechanism(s) outside the lungs. If LPTs were created by some mechanism(s) within the lung, abdominal pressures would have decreased as intrapulmonary pressure increased. This was not the case. If smooth muscle in the lung was contracting, lung pressure would increase, and coelomic pressure would decrease because the area within the coelom would increase due to the lung contracting. The pressure tracings (Fig. 3) between pulmonary and coelomic cavities leads me to conclude that skeletal, not smooth, muscle is clearly the primary mechanism behind the generation of the LPT.

II. How Depth Influences LPTs

LPTs were generated in 25-30% of all voluntary, unrestrained dives at all three water depths (Fig. 5). The frequency of LPTs occurring per hour remains almost constant between all three depths, and indicate that the hydrostatic pressure of dives in shallow to medium depths do not significantly alter LPT frequency. Based on the frequency of LPTs, about 6 per hour, being generated during diving, the actual occurrence of the LPT does not seem to be a random phenomenon. This rhythmic fluctuation in lung pressure during diving must presumably serve some physiological function. LPTs were generated even when smooth muscle was inactivated with high doses of atropine sulfate which acted to block the muscarinic receptors that activate smooth muscle contractions (Fig. 5). The frequency of LPTs decreased significantly in atropinized animals at the 10 cm depth, but not at the other depths. These data indicate that smooth muscle may play a small role in generating LPTs. Attempting to study LPTs by inactivation of skeletal muscle would be difficult since respiration is accomplished by maneuvers of specific skeletal muscle groups.

III. Influence of Dive Length on LPTs

Dives were separated into four different intervals to monitor the occurrence of LPTs during the duration of different length dives. In control animals the frequency of LPTs decreased with increasing dive length. That is, as the dive duration increased in length, the frequency of LPTs decreased. These data lead to the assumption that animals might predetermine how long their next dive will last and subsequently adjust the frequency of LPTs they produce. This could maximize the stirring of lung gases in relation to the length of the dive. A study performed by Burggren *et al.* (1988) described two distinct pattern changes in arterial blood PO_2 during diving in the turtle, *Chelodina longicollis*. In short dives arterial PO_2 decreased at the start of the dive, while longer dives would actually show slight increases in arterial PO_2. The study suggested that this turtle could alter arterial oxygen saturation during longer dive periods by periodically increasing pulmonary blood flow to transfer oxygen stored in lung gas into the blood.

Based on the two distinct patterns of selective arterial blood saturation and oxygen uptake during periods of apnea, it would seem that *C. longicollis* may plan out its dives. Since establishing that contraction of skeletal muscle is primarily responsible for creating LPTs, it must be taken into account that the contraction

of muscle (either smooth or skeletal) is an energy expenditure. Gases may be mixed slower in longer dives for overall conservation of energy and to conserve the rate of oxygen depletion from the lung. This is supported by the decrease in LPT frequency in these long dives. In short dives, the elevated frequency of LPTs leads to a more rapid mixing which would enhance the efficiency of gas exchange because the amount of oxygen at the respiratory surface would be inceased due to the prolonged disruption by LPTs of the fluid boundary layers found around gas exchange surfaces. Conserving oxygen would not be necessary in shorter dives, since the animal will soon surface and replenish its oxygen stores while also removing carbon dioxide from its lungs. The efficient management of oxygen uptake from the lung may be the critical factor regulating LPT frequency in these turtles.

IV. LPTs and Boundary Layer Disruption

Helium was used in these experiments to show the rate of lung gas distribution between laterally adjacent investments of a single lung and between both lungs. Helium redistribution between two lungs and within the same lung occurs approximately two fold faster when LPTs were present as opposed to when they were absent (Tables 1 and 2). Fluid dynamic boundary layers of different

concentrations form over or on gas exchange surfaces. The findings of the He experiments indicate that LPTs generated by lung contractions enhance mixing of gases within the same lung and between lungs so that stagnant boundary layers of both carbon dioxide and oxygen are disrupted. LPTs might directly cause mixing of lung gases via volume changes so that stagnant pockets do not accumulate in any particular chamber as well. This mechanism would definitely enhance pulmonary gas exchange in that unventilated regions or pockets of carbon dioxide and oxygen would not accumulate, hence gas exchange would be more evenly distributed throughout the lung.

A comparison between the means of the slopes generated in helium distribution occurring between the most posterior and anterior investments between animals which experienced LPTs and those which were not (Table 1) show that in the presence of LPTs He mixes nearly 2 fold faster as compared in trials where LPTs were absent. The distribution rate between the most anterior and posterior chambers in the same lung is very close to that of the distribution seen between lungs. Statistical analysis showed significance in both lateral chamber mixing and a mixing between lungs. Testing the distribution pattern of helium in this phase of the experiment was difficult because of the instability of cannulating and maintaining

a patent posterior lung investment for more than several trials. A more effective method may need to be explored in monitoring helium passage in the short distance occurring between the most posterior and anterior chambers.

V. Buoyancy

Jackson (1969) manipulated the specific gravity of turtles by the addition of weights (to increase specific gravity) and floats (to decrease specific gravity) to their shells. Jackson (1969) then occluded the cloaca in one group of turtles to see how they would react to the weights and floats. The experiment also entailed inducing decreases in lung volume to determine if water storage or release played a role in buoyancy. The findings of the experiment showed that *T. scripta* must have a large lung volume to compensate for the high specific gravity of its shell while in the water. The study also concluded that the cloacal bursa was the site for active short term water volume adjustments, and that the urinary bladder served in long term buoyancy adjustments. Milson and Johansen (1975) also showed that buoyancy was controlled by specific regulations in lung volume and that pulmonary smooth muscle plays an active role. The present study indicates that the contractions of skeletal muscle plays a

major role in lung compression which directly leads to volume distribution and displacement between lungs. Skeletal muscle could also play a role in controlling buoyancy by the same method. Future experiments might use X-ray techniques to photograph the lung during the course of a normal dive and in dives in which specific gravity of the turtle has been altered in order to determine how lung compressions (LPTs) are utilized in buoyancy regulation.

VI. Summary and Future Directions

The data gathered in this project support the hypothesis that transient lung pressure fluctuations are occurring during the dive of *Trachemys scripta elegans,* and serve to mix stagnant lung gases. The data also support the theory that these transient lung pressure fluctuations which occur during the dive, are caused by contractions of the respiratory (skeletal) muscle groups and/or by the striatum pulmonale, and possibly to a lesser extent by smooth muscle. In experiments in which helium redistribution between lungs, as well as distribution within the same lung was monitored, statistical analysis supported the theory that LPTs assist in stirring lung gases.

Further work in analyzing the phenomenon of lung pressure transients is required. The present study only

monitored a single lung for the study of LPTs. One additional study should be to monitor LPT generation during diving, in both lungs at the same time. Turtles may actively regulate (alternate) compressions of an individual lung or its compartment(s). This may further explain the findings of the Milson and Johansen (1975) study in which turtles could alter specific gravity during passive floatation experiments. Future considerations should be aimed at observing this phenomenon in different species of turtles. There are morphological variations between the different genera, and this study concentrated only on the North American red eared slider. Future work should also concentrate on monitoring LPTs at deeper depths, 1-5 meters, for example. A greater number of individuals should also be used where possible because the small number of animals used in this experiment may have masked other significant findings which were present but went unnoticed. Future experiments should also take careful account of the season in which observations are carried out in order to determine if and how LPTs are influenced by seasonality.

APPENDIX ONE

A brief summary of the discrepancies in the nomenclature of the red-eared slider, *Trachemys scripta elegans,* a widely distributed semi-aquatic pond turtle inhabiting the eastern regions of North America.

The reptilian order Chelonia [turtles and tortoises] represent an ancient group of reptiles which are not closely related phylogenetically to any of the other living reptilian orders. Turtles first appeared during the Triassic period (Halliday and Adler, 1981) and little is known about their origin. Today the Chelonia consists of over 240 species in 75 genera and thirteen families.

The history behind the taxonomy of the various species of aquatic pond turtles (Family: Emydidae) that live in the eastern regions of North America is not a simple one to follow. The extensive controversy stems mainly from biochemical, morphological, and paleontological misinterpretations (Siedel and Smith, 1986). The confusion in the taxonomy associated with the species used in this research project) dates back as far as the late 1700's. The complexity behind the naming process began with two genera. The genus *Testudo* which then included the painted turtles and the sliders, and the genus *Emys* which included the red eared slider (named for the brightly colored bar behind the eyes). This nomenclature lasted approximately twenty years before, J. G. Grey, a zoologist from the British Museum of Natural History in London in 1855, erected a new genus of turtle called *Pseudemys* (Siedel and Smith, 1986). The name *Pseudemys* referring to a false *Emys*. In 1857 a new genus

was erected and was named *Trachemys* (Agassiz, 1857), but would soon be "sunk" (owing to the Law of Priority which demands the oldest name be used). Numerous rearrangements would occur through the turn of century. During the 1930's the phenomenon of melanism caused great turmoil when it was found not to be a unique feature of a species hence, causing the "sinking" of several more names. The genus *Trachemys* was resurrected by M. E. Siedel and H. M. Smith (1986). This genus now includes five species and seventeen subspecies. The genus *Chrysemys* (painted turtles) contains one species with four subspecies and the genus *Pseudemys* (sliders) has 5 species with a highly fluctuating number of recognized subspecies. It will probably take several more years before the red eared slider is accepted as a member of the genus *Trachemys*, so until then, many will continue to consider it within the genus *Pseudemys*.

APPENDIX TWO

A summary of all the individual trials in which helium distribution experiments were conducted on the turtle, *Trachemys scripta elegans*. This includes helium redistribution occurring between lungs and the distribution of helium between the most posterior and anterior investments of a single lung.

Table 3: Shows All the Individual Trials Expressing Slopes (% Change in Helium/minute) and Statistics Generated by a Linear Regression Plotted Through Helium Concentrations Recorded Before the First Breath Taken By a Turtle, in an Individual Trial.

Helium Redistribution in the Opposite Lung

Turtle#	Trial#	slope	R value	R^2 value	LPT:+=(present) LPT:--=(absent)
26	2	0.025	0.95	0.903	+
26	3	0.278	0.978	0.956	+
26	6	0.063	0.951	0.904	--
26	7	0.037	0.878	0.771	--
27	2	0.028	0.921	0.848	+
27	3	0.091	0.791	0.848	--
29	1	0.061	0.751	0.567	+
29	2	0.048	0.911	0.830	--
29	5	0.05	0.711	0.504	--
30	1	0.1	0.792	0.628	+
30	3	0.102	0.533	0.248	--
30	4	0.564	0.948	0.842	+
30	5	0.012	0.918	0.843	--
31	1	0.147	0.907	0.822	+
31	2	0.176	0.757	0.574	--
31	3	0.444	0.884	0.784	--
31	4	0.491	0.912	0.831	+
31	5	0.544	0.918	0.843	+

Helium Distribution Through the Same Lung

Turtle#	Trial#	slope	R value	R^2 value	LPT:+=(present) LPT:--=(absent)
35	1	0.075	0.993	0.985	+
35	2	0.192	0.988	0.977	+
35	3	0.02	0.958	0.918	--
36	1	0.054	0.924	0.855	+
36	2	0.047	0.963	0.928	+
36	3	0.089	0.878	0.772	+
36	4	0.074	0.978	0.956	--
37	2	0.21	0.961	0.924	+
37	3	0.07	0.935	0.874	--
37	4	0.064	0.504	0.254	+
39	1	0.21	0.639	0.408	--
39	2	0.333	0.954	0.911	+
39	3	0.142	0.99	0.979	--
39	4	0.176	0.926	0.857	--
39	6	0.238	0.863	0.745	+
41	1	0.163	0.886	0.786	+
41	2	0.11	0.863	0.745	+
41	4	0.22	0.965	0.932	+
41	7	0.12	0.958	0.917	--

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