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The Pulmonary Circulation of the Chelonian Reptile: Morphology, Haemodynamics and Pharmacology

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Summary. 1. Blood pressures measured in lightly anaesthetized turtles (*Pseudemys scripta*) and tortoises (*Testudo graeca*) indicate that pressures throughout the ventricle are superimposable, but vascular impedances to blood flow in the pulmonary outflow tract and especially in the large extrinsic pulmonary arteries result in slightly lower pulmonary than systemic arterial systolic pressures in both species.

2. Pulmonary outflow tract impedance is increased by vagal stimulation and acetylcholine and decreased by adrenaline. However, the pulmonary outflow tract apparently contributes little to the overall pulmonary impedance changes which occur during intermittent breathing.

3. An analysis of pulmonary arterial impedance suggests that a large central arterial reservoir actively fills during systole and passively empties through a functionally single peripheral resistance during diastole. Morphological examination as well as in vitro compliance measurements and perfusion with drugs of the extrinsic pulmonary arteries corroborate these data by revealing a highly distensible central arterial reservoir nearly devoid of smooth muscle and vasomotor responses. The more distal pulmonary arteries are much less compliant, contain much smooth muscle, and show marked vasoconstriction in response to acetylcholine and vagal stimulation.

4. Data on pulmonary impedance, morphology and pharmacology are incorporated into a classic 'Windkessel' haemodynamic model of the chelonian pulmonary circulation.

Introduction

In contrast to mammalian and avian cardiovascular systems, the pulmonary and systemic circulations of the chelonian reptiles are perfused at largely similar

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systolic arterial blood pressures (White and Ross, 1966; Johansen et al., 1970; Shelton and Burggren, 1976). Nonetheless, the haemodynamics of the systemic and pulmonary circulations in the Chelonia still appear in many respects to be quite distinctive. Blood flow in the systemic arches of turtles is highly pulsatile, with little or no net flow occurring during diastole. However, blood flow in the central pulmonary circulation is maintained at a relatively high level throughout systole, and significant flow often continues for the duration of the diastolic period (White and Ross, 1966; Johansen et al., 1970; Shelton and Burggren, 1976). As another example, the rate of pressure decay during diastole is much greater in the chelonian pulmonary circulation than in the systemic circulation, and as a consequence (1) pulmonary pulse pressure is normally about twice as large as systemic pulse pressure and (2) ventricular blood ejection into the pulmonary circulation usually precedes ejection into the systemic circulation by some 60–100 ms (Steggerda and Essex, 1957; White and Ross, 1966; Shelton and Burggren 1976). These and other differences in the blood pressure and blood flow profiles of the systemic and pulmonary circulations of the chelonian reptiles have been attributed to both the highly distensible nature of the pulmonary arterial tree and the fact that systemic resistance tends to be somewhat elevated compared to pulmonary resistance (Johansen et al., 1970; Shelton and Burggren, 1976). Another difference of fundamental importance between the pulmonary and systemic circulations of the Chelonia is the relative magnitude of flow variations that accompanies apnoea and lung ventilation. Unlike the mammalian or avian cardiovascular system, the functional continuity of the ventricular chambers of the chelonian ventricle during at least periods of apnoea (White and Ross, 1966) or throughout intermittent breathing as alternatively suggested by Shelton and Burggren (1976) and Burggren, Glass and Johansen (in preparation), essentially couples the pulmonary and systemic circulations in parallel with the ventricular perfusion pump. Hence, the largest proportion of the total cardiac output will be distributed to that circulation which offers the lower impedance to blood flow. In the chelonian reptiles selective perfusion of the pulmonary or systemic circulation is achieved to a large extent by alterations in pulmonary impedance, while the impedance of the systemic circulation is maintained at a more constant level (Shelton and Burggren, 1976). The locus of this impedance change within the pulmonary circulation has not been unequivocally established. White (1970) suggests that the pulmonary vascular bed itself is of major importance, and certainly the potential for vasomotor activity in the blood vessels within the reptilian lung has been well established (Maar, 1902; Krogh, 1910; Luckhardt and Carlson, 1921; Berger, 1973). However, both morphological and experimental evidence indicates that vasculature proximal to that actually within the lung may also contribute to pulmonary impedance changes during intermittent lung ventilation (Woodbury and Robertson, 1942; March, 1961; Berger, 1973).

This paper thus investigates the nature and significance to the overall circulation of vasomotor responses in specific regions of the pulmonary arterial vasculature of *Pseudemys scripta* and *Testudo graeca* during both in vitro perfusion and in the intact pulmonary circulation. A morphological basis for such vasomotor responses as have occurred is established by a histological examination

of the pulmonary arterial tree. In the light of these and other data on arterial wall distensibility and pressure pulse wave velocity, the dynamic nature of blood pressure/flow relationships which have been previously determined in the pulmonary arterial circulation of the turtle and the tortoise can now be more clearly interpreted in terms of a simple lumped parameter electrical model of the pulmonary circulation.

Methods

Forty-five healthy specimens of *Pseudemys scripta* and *Testudo graeca* weighing between 1.0 and 1.6 kg were used for this investigation. All experimental work was performed at 20 °C.

Light Microscopy

Tissue removed from the pulmonary arteries of freshly killed animals was fixed in Bouin's fluid and then embedded in paraffin wax. Iron haematoxyline and eosin were used to stain the 8 μ thick sections cut from the paraffin wax blocks. The sections were examined and photographed in a Zeiss Universal microscope.

In vivo Blood Pressure and Blood Flow Measurements

Turtles and tortoises were anaesthetised for surgery by cold torpor (White and Ross, 1966). Surgical techniques of pressure cannulae and flow probe implantation, and the pressure and flow monitoring instrumentation and their calibration procedures have already been described elsewhere in considerable detail (Shelton and Burggren, 1976). Briefly, the common pulmonary artery of turtles and tortoises was non-occlusively cannulated (20 cm nylon 00) in a downstream direction just distal to the pulmonary valves. The left proximal pulmonary artery was similarly cannulated immediately above the arterial ligament and the tip of a third cannula was introduced through a needle stab wound into the cavum pulmonale of the ventricle. The cannulae were filled with heparinized saline and 200 i.u./kg body weight of heparin were injected into each animal at the beginning of the experiments. The implanted cannulae were attached to Bio-Tech BT70 or Sanborn 267B pressure transducers whose outputs were displayed on a Sanborn 966 chart recorder writing on rectangular co-ordinates. Frequency and damping characteristics of the pressure cannulae were adequate to record all pressure transients without significant amplitude or phase lag error. Pulmonary blood flow measurements were made with a Biotronex BL410 electromagnetic blood flow meter and displayed on the Sanborn 6-channel chart recorder. After the completion of surgery, the animals were warmed to room temperature and light anaesthesia was administered with Halothane vapour. The level of the anaesthesia in the experiments which followed was adjusted to the level where voluntary breathing was just maintained.

In vitro Arterial Distension

Pressure/volume relationships in various regions of the pulmonary arterial tree were determined in vitro from the rate of arterial pressure rise measured during a constant rate of arterial distension with air-equilibrated saline or solutions of acetylcholine or adrenaline in saline. A PE 200 cannula tied into either the entire extrinsic pulmonary arterial tree or into a 10 mm length of excised artery was connected to a Braun injection device (Fig. 1, top). A second cannula tied into the preparation was connected to a Sanborn 267B pressure transducer, whose output was recorded on a Devices 2-channel recorder. Before saline injection was initiated the entire preparation was gently collapsed and emptied by the application of slight negative pressure to the cannulae. Thus, the volume of the preparation was effectively negligible when distension began. Distension was

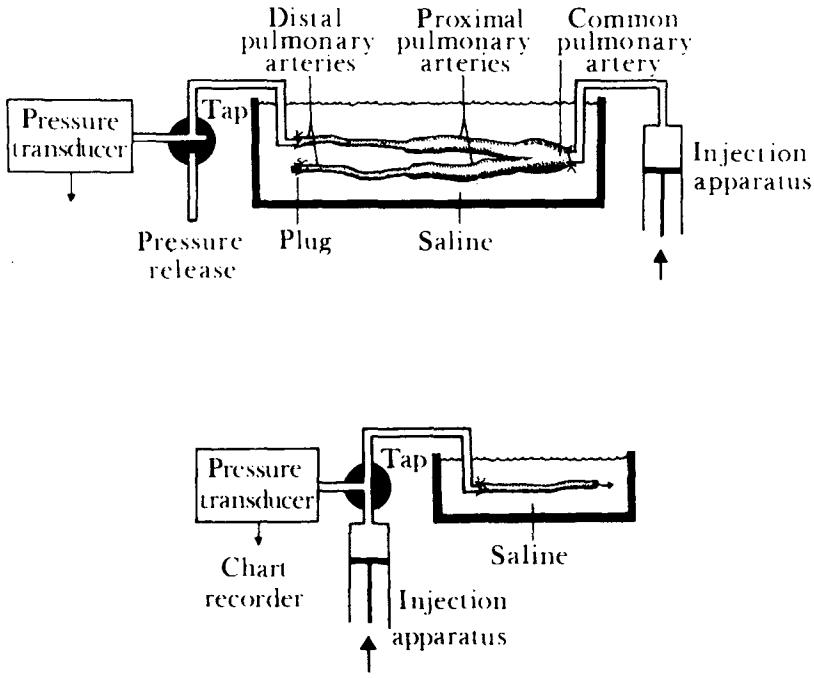

Fig. 1. Diagrammatic representation of the experimental apparatus used for the in vitro measurement of (top) arterial distensibility and (bottom) vasomotor responses in the extrinsic arterial pulmonary vasculature of *Pseudemys* and *Testudo*

continued until an arterial pressure of 60–75 cm H_2O was attained, whereupon the preparation was immediately allowed to empty.

Perfusion of the Pulmonary Vasculature

The in vitro perfusion pressure of the proximal or distal segment of the pulmonary artery was measured via a 'T' junction inserted into the perfusion line connected to a Sanborn 267B pressure transducer (Fig. 1, bottom). Perfusion with saline or solutions of acetylcholine or adrenaline in saline was at a rate of 2.0 ml/min. In some freshly pithed animals the central tip of the distal pulmonary artery was occlusively cannulated in a downstream direction in situ. The distal artery was severed at its point of entry into the lungs, and saline perfusion begun. Nerve fibres separated from the ipsilateral vagus nerve in the neck were laid on a pair of silver wire electrodes connected to a Grass S4 stimulator, and the effect of vagal stimulation on the perfusion pressure in the in situ preparation was recorded.

Responses of the pulmonary outflow tract of freshly pithed animals to drugs and nerve stimulation were also investigated. The pulmonary valves of the excised outflow tract were surgically removed and the base of the common pulmonary artery was occlusively cannulated in an upstream direction and attached to the perfusion and pressure recording instruments. Silver wire electrodes connected to the stimulator delivered stimulating pulses (5 pulses/min) directly to the cavum pulmonale, thus inducing rhythmic contraction of the entire preparation during retrograde perfusion. Outflow tract perfusion was also performed in situ in freshly pithed animals and alterations in perfusion pressure in response to stimulation of nerve fibres separated from the left or right vagus trunk in the neck were measured.

In vivo perfusion of the intact circulation of one lung during voluntary, intermittent breathing in lightly anaesthetized animals was performed by occlusively cannulating (PE 200) the left pulmonary artery in a downstream direction. The left pulmonary vein was occlusively cannulated in an upstream direction to provide venous drainage. Perfusion of the intact left lung with heparinized, air-equilibrated saline was then begun at a rate of 2.0 ml/min. Dissection after the experiments

were terminated revealed no pulmonary oedema. Lung ventilation was monitored using a pneumotach screen which was fitted in the finger of a rubber glove and placed over the animal's head (Burggren, 1975).

Results

Morphology of the Pulmonary Arteries

Marked morphological variation between different regions of the chelonian pulmonary arterial tree has long been appreciated (see Brenner, 1883, as quoted in O'Donoghue, 1916; Kock, 1934). Structurally, the 'extrinsic' pulmonary vasculature central to the lung parenchyma can be categorized into three distinct regions; the pulmonary outflow tract including the overlying bulbus cordis; the proximal vasculature, which consists of the large elastic common pulmonary artery and the left and right proximal pulmonary arteries which extend to the level of the arterial ligament (ligament of Botallus); and the distal vasculature, composed of the pulmonary arteries from the arterial ligament to the lungs. (These regions of the pulmonary arterial vasculature are illustrated diagrammatically in Fig. 1, top.) Although in some of the reptiles the bulbus cordis has disappeared with the evolution of a separate pulmonary circulation, the bulbus cordis in the Chelonia still persists on the pulmonary artery as a small band of cardiac muscle extending around its base and onto the left medial edge of the left aorta, underlying the 1–2 mm thick cardiac muscle band of the bulbus cordis and constituting the innermost layer of tissue lining the bulbar region of the pulmonary outflow tract is a discrete, 1 mm thick layer of smooth muscle fibres. Although the cardiac muscle of the bulbus cordis is evident mainly on the ventral surface of the common pulmonary artery as it emerges from the ventricle, the innermost layer of smooth muscle fibres wraps circularly around the entire base of the pulmonary artery. In *Testudo* the cardiac muscle component of the bulbus cordis is much less evident externally than in *Pseudemys*, although the smooth muscle component is equally represented in both genera.

The cardiac and smooth muscle fibres of the pulmonary outflow tract extend out into the base of the common pulmonary artery for 2–3 mm, but are more distally replaced by arterial wall components characteristic of the proximal pulmonary vasculature (Fig. 2A, C and D). Lining the arterial lumen in this region is a thin and indistinct endothelial layer, or tunica interna. The 200–300 μ thick tunica media contains oval, non-elongated smooth muscle fibres usually orientated in a circular or helical direction around the artery lumen. Interspersed throughout the smooth muscle cells, however, are many thin layers of collagen and elastic fibres which externally form the tunica adventitia, the 100–400 μ thick sheath of connective tissue encompassing the artery. An unusual feature of the proximal vasculature is the presence (in both fresh and fixed material) of numerous 50–250 μ long projections of the arterial endothelium which radiate at various angles out into the artery lumen, especially distal to the bifurcation of the common pulmonary artery (Fig. 2A).

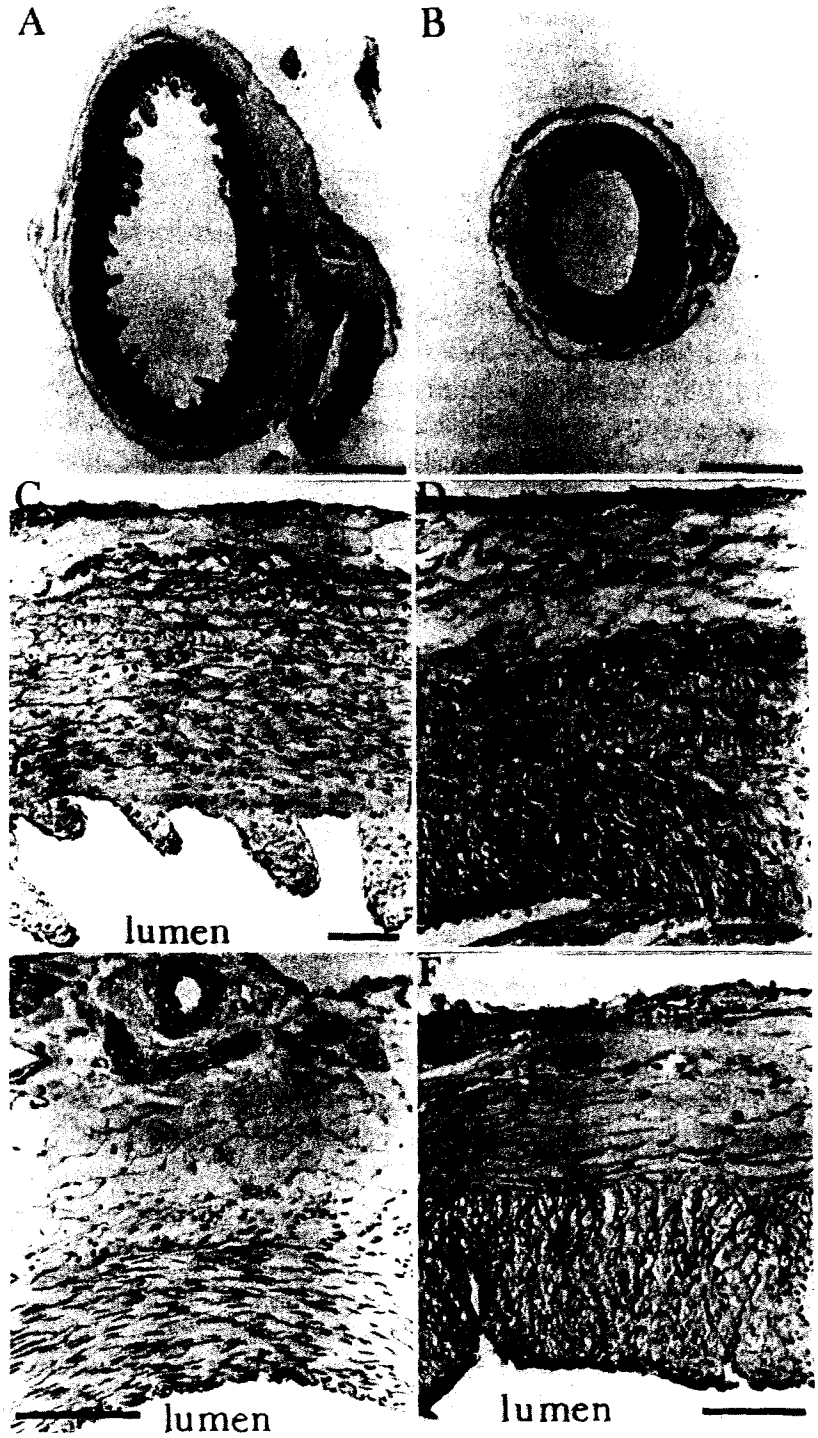

Fig. 2A–F. Transverse section of the left pulmonary artery of *Pseudemys scripta* **A** above and **B** below the arterial ligament. These regions of the pulmonary artery have been designated as the proximal and the distal pulmonary arterial vasculature, respectively. The scale in **A** and **B** is indicated by a 1.0 mm bar. The smooth muscle fibres in the tunica media of the proximal pulmonary artery are relatively disperse in both **C** transverse section and **D** sagittal section. In the distal pulmonary artery, however, the smooth muscle fibres are concentrated into a highly organized tunica media when viewed **E** in transverse section and **F** in sagittal section. The scale in **C–F** is indicated by a 100 μ bar

The beginning of the distal vasculature is marked by an abrupt decrease in pulmonary arterial diameter from approximately 3.0–4.5 mm above the arterial ligament to only 1.5–2.5 mm immediately below it (Kock, 1934; Fig. 2A and B). The lumen of the distal artery is only 0.6–0.8 mm in diameter although the 0.25–0.4 mm thick arterial wall is comparable in thickness to that of the proximal vasculature (Fig. 2D and 2F). Unlike the proximal arteries, the distal arteries possess a very discrete tunica media some 100–200 μ thick composed almost exclusively of large numbers of elongated smooth muscle cells orientated in a circular direction around the artery lumen (Fig. 2E and F). The tunica adventitia of the distal arteries, though consisting of the same structural elements as the proximal vasculature, is considerably thicker in relation to total diameter and contains numerous small blood vessels and nerve fibres, as Weathers and White (unpublished) have similarly observed.

Haemodynamics of the Pulmonary Circulation

1. Blood Pressure and Blood Flow

Pressure waveforms recorded at the central end of the common pulmonary artery during normal ventricular ejection in voluntarily breathing but lightly anaesthetized animals were nearly identical to those recorded in the cavum pulmonale and cavum venosum of the ventricle (Fig. 3A). The slight pressure gradient of 0.5–1.0 cm H_2O which normally developed across the pulmonary outflow tract particularly during late systole was greatly augmented, however, by stimulation of the left or right vagus nerve (3 V, 15 Hz, 5 ms duration) (Fig. 3B). In some instances, a pressure gradient of 7–10 cm H_2O over a length of just 1 cm from the cavum pulmonale to the common pulmonary artery prevailed during vagal stimulation.

Pressure waveforms recorded at the peripheral end of the proximal pulmonary artery were nearly superimposable on central common pulmonary artery pressure waveforms throughout the cardiac cycle, although often both systolic and diastolic pressures were approximately 0.5 cm H_2O lower at the downstream pressure recording site (Fig. 3A). This was the case during all experimental conditions, including vagal stimulation, even though the mean blood pressure sometimes underwent considerable change.

Ventricular and pulmonary arterial pressures remained as described above during both apnoea and lung ventilation in these lightly anaesthetized animals.

In some experiments the tip of the movable cannula was carefully advanced downstream into the distal pulmonary artery. Whenever a cannula is passed down the length of an artery the reduction in cross-sectional area of the lumen by the presence of the cannula itself will be manifested in an artificially produced fall in blood pressure measured at the cannula tip. While the absolute levels of blood pressure recorded in the distal pulmonary artery (Fig. 3C and D) are thus highly artificial, the experiment is nonetheless informative, for only by a reduction in distal pulmonary artery diameter can the measured pressure gradient, whether real or artificial, be increased in such an experiment. When the vagus nerves were stimulated at the level of the neck, the pressure drop

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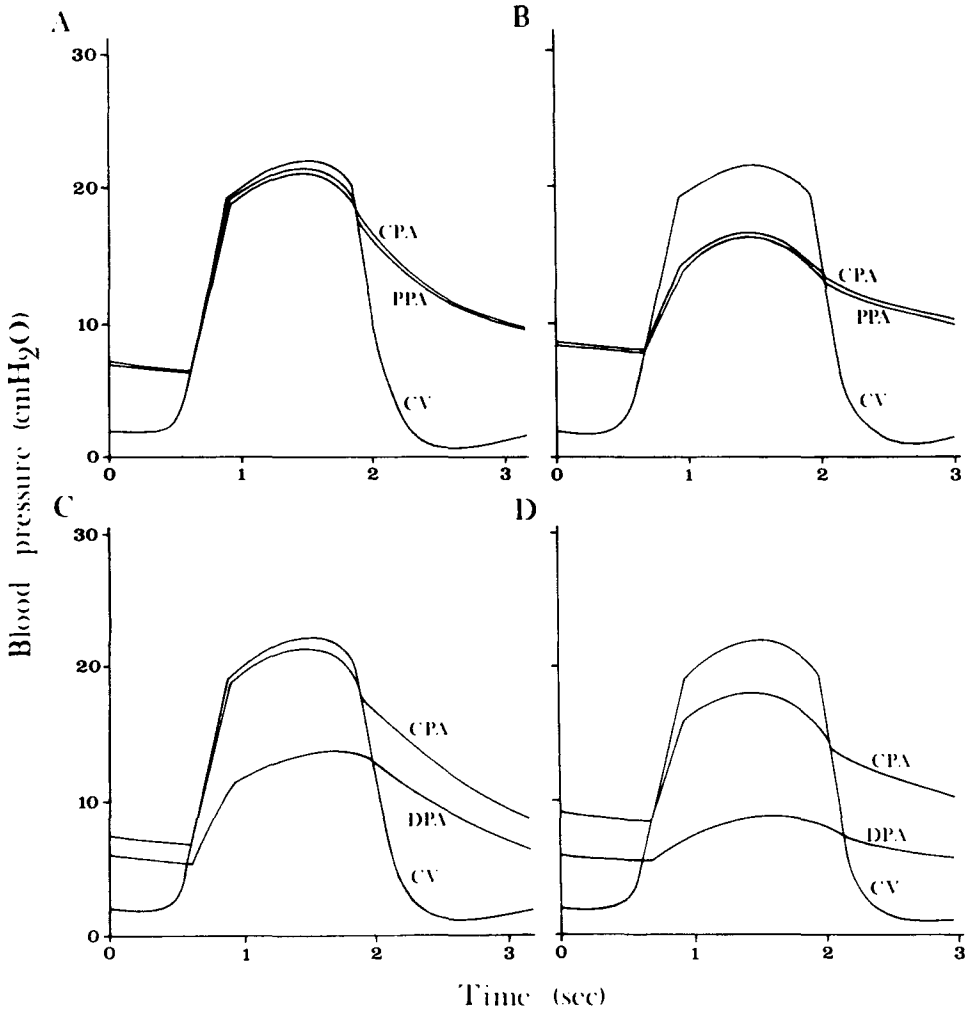

Fig. 3A–D. Superimposed blood pressure waveforms recorded simultaneously in the pulmonary arterial circulation of a lightly anaesthetized *Pseudemys scripta* under control conditions (**A** and **C**) and during efferent vagal stimulation at 3 V, 15 Hz (**B** and **D**) *CP*, cavum pulmonale; *CPA*, common pulmonary artery; *DPA*, peripheral end of the distal pulmonary artery; *PPA*, peripheral end of the proximal pulmonary artery

along the distal pulmonary artery increased enormously (Fig. 3C and D). In some experiments vagal stimulation caused the distal artery to vasoconstrict around the cannula so tightly that the measured pressure fell to 'pulmonary wedge' levels, indicating complete arterial occlusion proximal to the cannula tip.

The pressure wave 'foot', the point at which arterial pressures reflect valve opening and the steep rise of the waveform begins, was recorded at the peripheral end of the proximal pulmonary vasculature only 10–15 ms later than at the central end. The pressure wave foot was also quite distinct in the distal pulmonary arteries, even during vagal stimulation (Fig. 3C and D). There was approximately a 30 ms delay in these pressure transients recorded at two sites 50 mm apart at the central end of the common pulmonary artery and at the peripheral end of the distal artery, indicating an overall pressure pulse velocity of approximately 1.7 m/s within the extrinsic pulmonary arteries of *Pseudemys*.

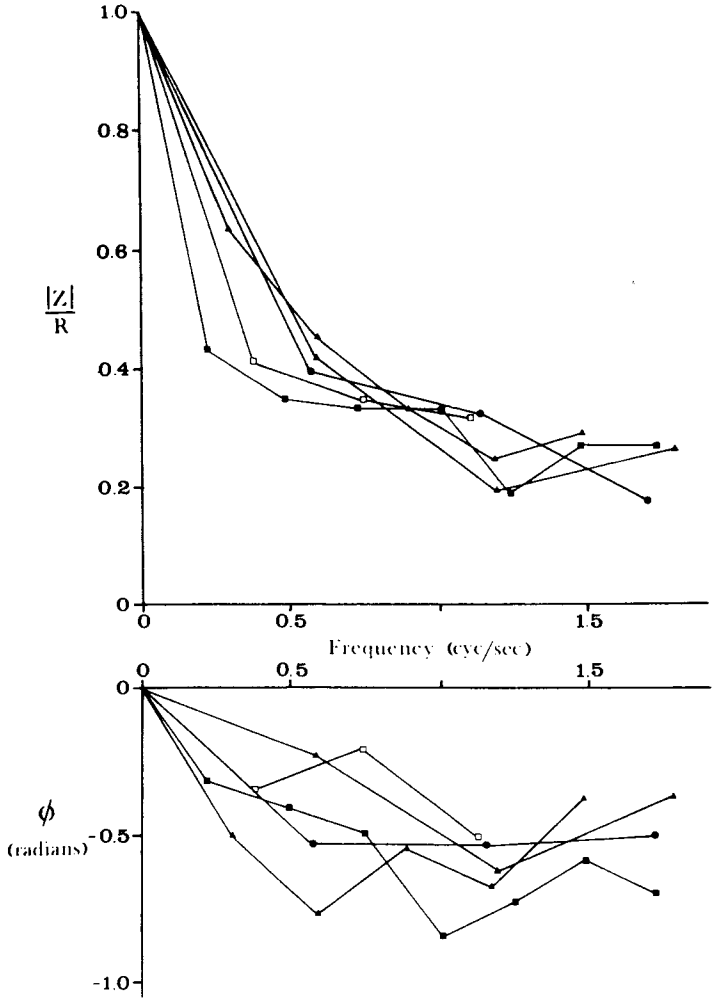

Fig. 4. Impedance and phase computed for the pulmonary arterial circulation of 4 *Pseudemys scripta* (closed symbol) and 1 *Testudo graeca* (open symbols) during lung ventilation

2. Pulmonary Artery Impedance

Records of pulmonary artery blood pressure and flow during lung ventilation in 4 *Pseudemys* and 1 *Testudo* were subjected to a Fourier analysis to determine pulmonary artery impedances. The impedance modulus of each turtle and tortoise has been normalized by dividing by the impedance at 0 frequency (peripheral resistance) to allow comparison between different animals. Impedance modulus in the pulmonary circulation of all 5 animals decreased rapidly and at a similar rate between a harmonic frequency of 0 and 0.5 Hz (Fig. 4). As the frequency increased further, the impedance generally decreased much less rapidly, and in some of the animals did not change significantly above a frequency of 0.75–1.0 Hz. Impedance phase, negative when flow leads pressure and positive when pressure leads flow, increased from 0 to approximately 0.5 radians at the first to second harmonic frequency. Impedance phase varied more from animal to animal than did modulus, and showed relatively large fluctuations at higher frequencies. These fluctuations, as well as those in impedance phase, were apparently completely random.

Impedance relationships in the pulmonary circulation were markedly different during apnoea. Impedance modulus increased at all harmonic frequencies during apnoea, while the impedance phase showed a concomitant decrease. Pulmonary vasomotor responses responsible for these impedance fluctuations, as well as regional structural properties of the pulmonary arterial vasculature which may also influence pulmonary blood flow, are described below.

Distension of the Pulmonary Vasculature

1. Pressure/Volume Curves

Pressure in the complete extrinsic pulmonary vasculature of both *Pseudemys* and *Testudo* rose in an almost linear fashion with increasing arterial volume during distension, even at pressures well above normal pulmonary systolic pressures (Fig. 5A). However, many successive distensions of the same preparation produced progressive, non-reversible shifts towards larger arterial volume. These changes were attributable mainly to changes in the properties of the common pulmonary rather than the distal pulmonary artery. Because of this phenomenon all data for analysis whenever possible were taken from the first in a series of distensions. Although distinct differences existed between the curves obtained from the different regions of the chelonian pulmonary arteries, each contained the same basic components of a small slope of pressure rise as very extensible elastic fibres were first stretched (and smooth muscle fibres, if unexcited, which have a similar elasticity), followed by a final steep slope of pressure rise as relatively indistensible collagenous tissue became engaged (Fig. 5B). The pressure/volume curves of 10 mm long pieces of the distal artery were displaced substantially to the left of the curves obtained from the same length of either the proximal vessel or the aorta. The distal artery is of much smaller diameter than either the proximal artery or aorta, and pressure within it reached the maximum experimental pressure at a much smaller volume.

2. Pulmonary Artery Distensibility

The % distensibility of the entire extrinsic pulmonary arteries is approximately twice as great in *Pseudemys* as in *Testudo* (Table 1). However, the increase in total extrinsic pulmonary volume during the rise from in vivo diastolic to systolic pressure is approximately 0.65–0.70 ml in both species, since lower systolic pressures are generated in the more distensible pulmonary vasculature of the turtle (Shelton and Burggren, 1976). The patterns of distensibility variation between the different regions of the pulmonary arteries from both *Pseudemys* and *Testudo* are quite similar. The proximal artery, for example, is 200–300% more distensible than the distal artery in both the turtle and the tortoise (Table 1).

As a consequence of its relatively great distensibility and large diameter, the common pulmonary artery alone in both *Pseudemys* and *Testudo* accounts

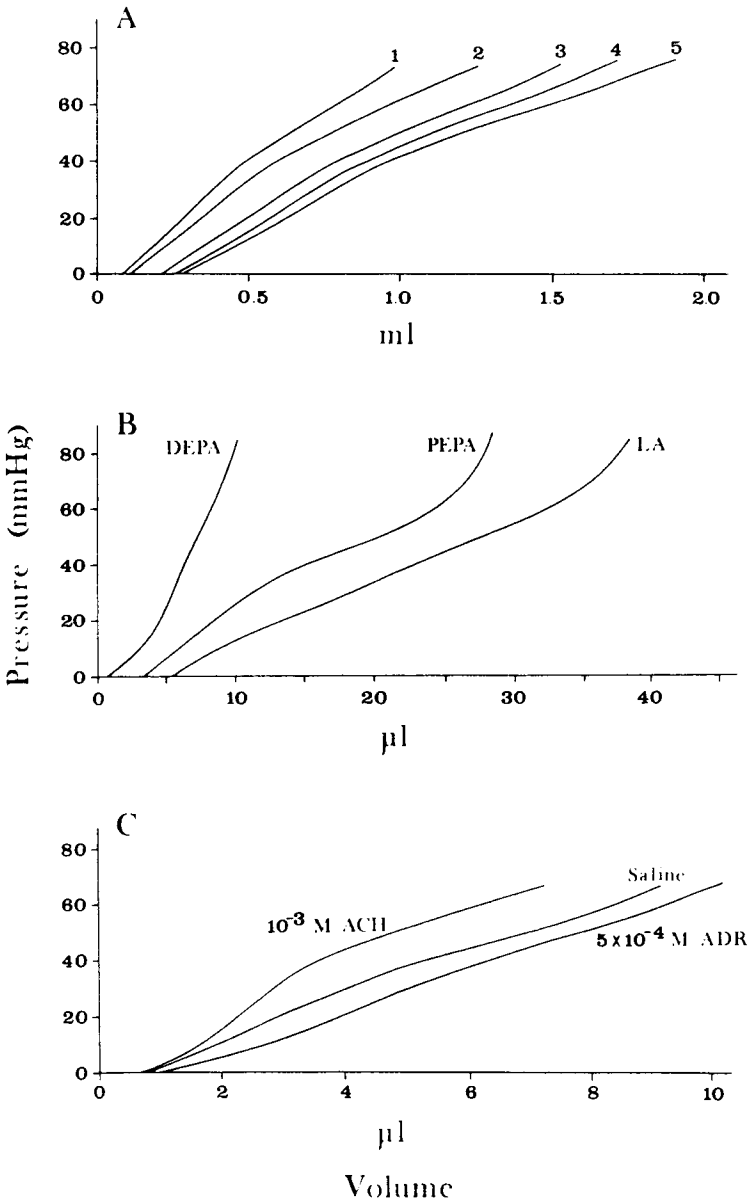

Fig. 5. **A** Pressure/volume curves determined in vitro for the entire extrinsic pulmonary arterial vasculature of *Pseudemys scripta*. The curves numbered 1–5 were determined from successive distensions of the same preparation. **B** Pressure/volume curves determined in vitro for 10 mm lengths of the distal (DEPA) and proximal (PEPA) extrinsic pulmonary artery and the left aorta (LA) of *Testudo graeca*. **C** Pressure/volume curves determined in vitro for a single 10 mm length of distal pulmonary artery from *Testudo graeca* during distension with saline, acetylcholine and adrenaline

for the largest proportion of the absolute volume distensibility (A.V.D.) of the extrinsic pulmonary arteries (Table 1). Even though occupying half of the length of the extrinsic vasculature, as a consequence of their low distensibility and small diameter, only 10% of the absolute volume distensibility of the entire extrinsic pulmonary arteries can be attributed to the distal arteries.

The % distensibility of the aorta approximates that of the distal rather than the proximal pulmonary vasculature (Table 1). However, because of a

Table 1. Arterial dimensions and distensibility measured in vitro in the extrinsic pulmonary arterial vasculature and the left aorta of 8 *Pseudemys scripta* and 9 *Testudo graeca* (weight range 1.0–1.6 kg). All values presented are means ± 1 standard error. Values for *Testudo* are in brackets

Calculations of arterial wall distensibility are based on the in vivo range of pulmonary blood pressures for *Pseudemys* (35/15 cm H_2O) and *Testudo* (50/15 cm H_2O), according to values given by Shelton and Burggren (1976). % distensibility was calculated as $\Delta V/V\Delta P \cdot 100$, where ΔV is the change in arterial volume in µls (diastolic to systolic pressure), V is the initial arterial volume in µls (at diastolic pressure), and ΔP is the change in pressure in cm H_2O (pulse pressure). Absolute volume distensibility was calculated as $\Delta V/\Delta P$

	Length[a] (mm)	Initial volume (µl)	% Distensibility (%/cm H_2O)	Absolute volume distensibility (µl/cm H_2O)	Total A.V.D.[c] (µl/cm H_2O)	% of total A.V.D.
Entire extrinsic arterial pulmonary vasculature	57 ± 2 (60 ± 5)	308 ± 60 (356 ± 56)	11.0 ± 0.7 (5.4 ± 0.5)	32.5 ± 5.7 (19.6 ± 2.0)	32.5 ± 5.7 (19.6 ± 2.0)	100
Common pulmonary artery	9 ± 1 (10 ± 1)	– –	– –	– –	22.0 ± 5.2 (11.3 ± 1.3)	64 ± 7 (58 ± 3)
Proximal extrinsic pulmonary artery	21 ± 1 (23 ± 1)	25 ± 7[b] (32 ± 5)	7.2 ± 1.0 (4.2 ± 0.3)	1.7 ± 0.2[b] (1.3 ± 0.1)	7.6 ± 0.9 (5.0 ± 1.0)	27 ± 2 (29 ± 3)
Distal extrinsic pulmonary artery	27 ± 2 (27 ± 2)	21 ± 4[b] (20 ± 3)	2.6 ± 0.4 (2.6 ± 0.2)	0.5 ± 0.1[b] 0.5 ± 0.1)	3.0 ± 0.6 2.4 ± 0.5)	10 ± 1 (13 ± 1)
Left aorta	– –	33 ± 4[b] (53 ± 7)	4.2 ± 1.0 (2.8 ± 0.2)	1.5 ± 0.2[b] (1.5 ± 0.2)	– –	– –

[a] Length of one side only of the paired proximal and distal arteries

[b] Data for 10 mm lengths of artery excised from the indicated regions

[c] Total A.V.D. of paired distal proximal arteries = (A.V.D. of 10 mm length of artery in µls ÷ 10) · (Length of extrinsic artery in mm) · (2)

substantially larger diameter, the absolute volume distensibility of a 10 mm length of aorta is more similar to that of the same length of proximal pulmonary artery.

Proximal artery distensibility was altered by neither acetylcholine nor adrenaline in any dosage, with the exception of a single preparation in which adrenaline produced a slight decrease in this parameter. However, the pressure/volume curve of the distal pulmonary vasculature was consistently affected by both acetylcholine and adrenaline (Fig. 5C). At doses eliciting maximal responses, acetylcholine (10^{-4} M) reduced distal distensibility from control levels while adrenaline ($5 \cdot 10^{-5}$ M) increased this parameter (Fig. 5C).

Vasomotor Responses of the Pulmonary Vasculature

1. The Pulmonary Outflow Tract

Two distinct groups of musculature operate independently to influence pulmonary outflow tract impedance. Vasomotor activity of the vascular smooth muscle

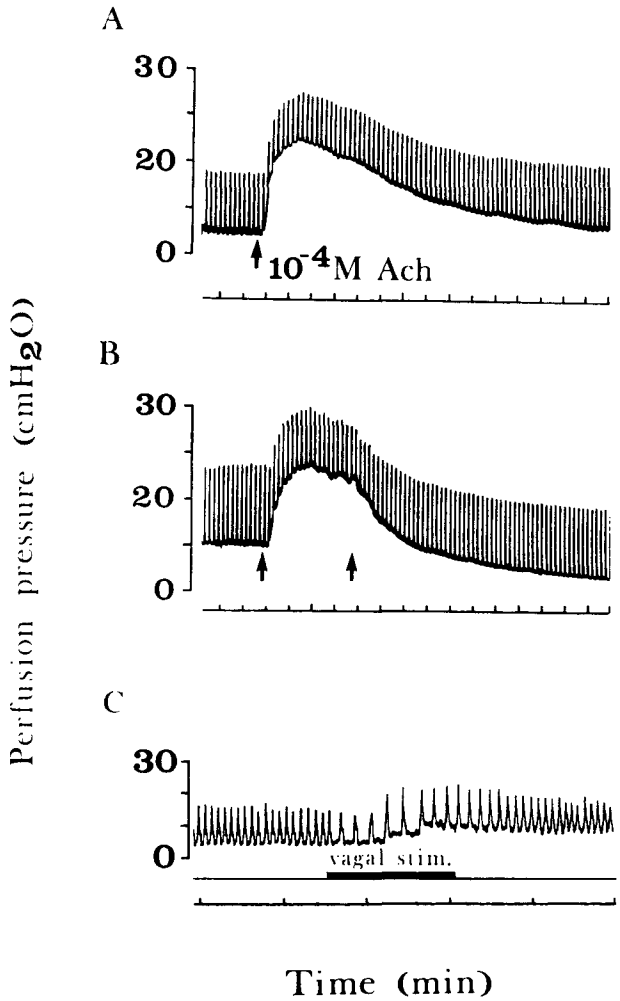

Fig. 6A–C. Changes of the perfusion pressure of the pulmonary outflow tract of *Pseudemys scripta* produced in vitro by **A** 10^{-4} M acetylcholine and **B** 10^{-5} M acetylcholine (first arrow) followed after 4 min by 10^{-4} M adrenaline (second arrow). The effect of efferent vagal stimulation (3 V, 15 Hz) on the in situ perfusion pressure of the pulmonary outflow tract of a pithed *Testudo graeca* is shown in **C**

lining the outflow tract under the *bulbus cordis* produces a change in outflow tract diameter which develops over a period of several seconds. The tone of this smooth muscle will thus directly influence outflow tract impedance in vivo whenever the pulmonary valves are open and blood is flowing into the pulmonary circulation. Unlike the smooth muscle layer, the *bulbus cordis* undergoes a single rapid contraction and relaxation during the later part of each systole. It also serves to reduce pulmonary outflow tract diameter, but produces a much more transient effect on outflow impedance. When smooth muscle vasoconstriction has caused a relatively long-term reduction in outflow tract diameter, contraction of the *bulbus cordis* will produce an even greater transient impedance increase in the pulmonary outflow tract during late systole (Fig. 6).

In vitro perfusion of the pulmonary outflow tract with 10^{-5} M acetylcholine caused a large increase in the 'baseline' perfusion pressure, that pressure evident in between the transient resistance increases produced by successive *bulbus cordis*

contractions (Fig. 6A). This vasoconstrictor response of the smooth muscle lining the outflow tract was blocked by pretreatment of the preparation with 10^{-6} M atropine. 10^{-4} M adrenaline had little effect on untreated preparations, but decreased the baseline perfusion pressure of preparations with a cholinergically elevated vasomotor tone (Fig. 6B). Pre-treatment of the preparation with propranolol, an adrenergic β-blocker, abolished this adrenergic vasodilatory response. Peripheral stimulation of fibres separated at the level of the neck from the right vagus nerve produced a significant increase in the baseline perfusion pressure of the outflow tract in situ (Fig. 6C). Pre-treatment of the preparation with 10^{-6} M atropine abolished all effects of vagal stimulation. Stimulation at the level of the neck of sympathetic nerves which produced a positive chronotropic and inotropic cardiac effect did not influence the perfusion pressure of the pulmonary outflow tract.

2. Proximal Pulmonary Arteries

Proximal pulmonary arteries excised from 8 animals were cannulated and then perfused with solutions of acetylcholine (10^{-5} M) or adrenaline (10^{-5} M). Generally, no vasomotor activity as manifested by changes in perfusion pressure was observed in the proximal pulmonary arteries, although in a single experiment a short-lived vasodilation of extremely small magnitude was observed during perfusion with an adrenaline solution. Neither parasympathetic nor sympathetic nerve stimulation had any effect on the resistance of the perfused proximal artery.

3. Distal Pulmonary Arteries

Acetylcholine invariably produced a marked response in the distal vasculature of the 10 *Pseudemys* and *Testudo* examined (Fig. 7A). Significant increases in arterial resistance were observed with doses of acetylcholine as low as 10^{-7} M, although high doses of $5 \cdot 10^{-5}$ M to 10^{-4} M were often required to produce maximal increases in the resistance of the preparation. The maximal increase in distal artery resistance, in the order of 300–500%, occurred within 1 to 2 min of the initiation of acetylcholine perfusion, but was relatively short-lived, for a decrease in the resistance to an equilibrium closer to control levels then usually developed (Fig. 7A). Adrenaline invariably produced a decrease in the resistance of the perfused distal pulmonary preparation. However, $5 \cdot 10^{-5}$ M to $5 \cdot 10^{-4}$ M adrenaline, a dosage found to produce maximal responses, caused a mean reduction in control resistance of only 15–20% in both species. If the tone of the preparation was first increased by acetylcholine, however, the full vasodilatory potential of adrenaline could be realized (Fig. 7B). As with the cholinergic constrictor response, the maximum adrenergic dilator response occurred within 1–2 min of adrenaline exposure.

Stimulation of nerve fibres separated from the ipsilateral vagus nerve produced marked increases in the in situ perfusion pressure of the distal pulmonary artery of pithed animals (Fig. 7C). Nerve stimulation was usually not so effective as acetylcholine perfusion in producing vasoconstriction. Petreatment of the preparation with 10^{-6} M atropine abolished vasoconstriction resulting

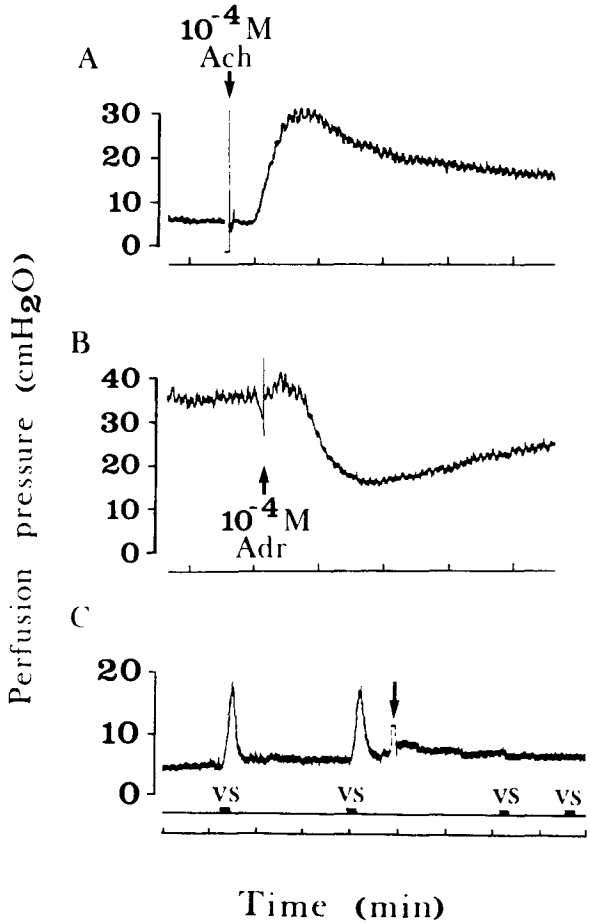

Fig. 7A–C. Changes of the perfusion pressure of the distal pulmonary artery of *Testudo graeca* produced in vitro by **A** acetylcholine and **B** adrenaline. In **B** the tone of the preparation had been elevated by pretreating with 10^{-4} M acetylcholine before the start of the record. Arrows indicate the initiation of drug perfusion. **C** shows the effect of left vagal efferent stimulation (*VS*) (3 V, 15 Hz) on in situ perfusion pressure of the left distal pulmonary artery of a freshly pithed *Pseudemys scripta*. The preparation was perfused with a solution of 10^{-6} M atropine after the arrow

from perfusion with acetylcholine or from vagal stimulation. The perfusion pressure was unaffected during stimulation of sympathetic fibres in the neck which were responsible for producing positive inotropic and chronotropic cardiac effects.

The resistance of the outflow tracts of the aortae and the brachiocephalic artery was in no instance influenced by acetylcholine, adrenaline, or sympathetic and parasympathetic nerve stimulation. Perfused lengths of either the left or right aorta exhibited cholinergic vasodilation and adrenergic vasoconstriction of relatively small magnitude.

In vivo Pulmonary Vasomotor Responses Associated with Intermittent Breathing

Saline perfusion at a rate of 2.0 ml/min produced a perfusion pressure in the 'intact' pulmonary circulation of lightly anaesthetised, intermittently breathing

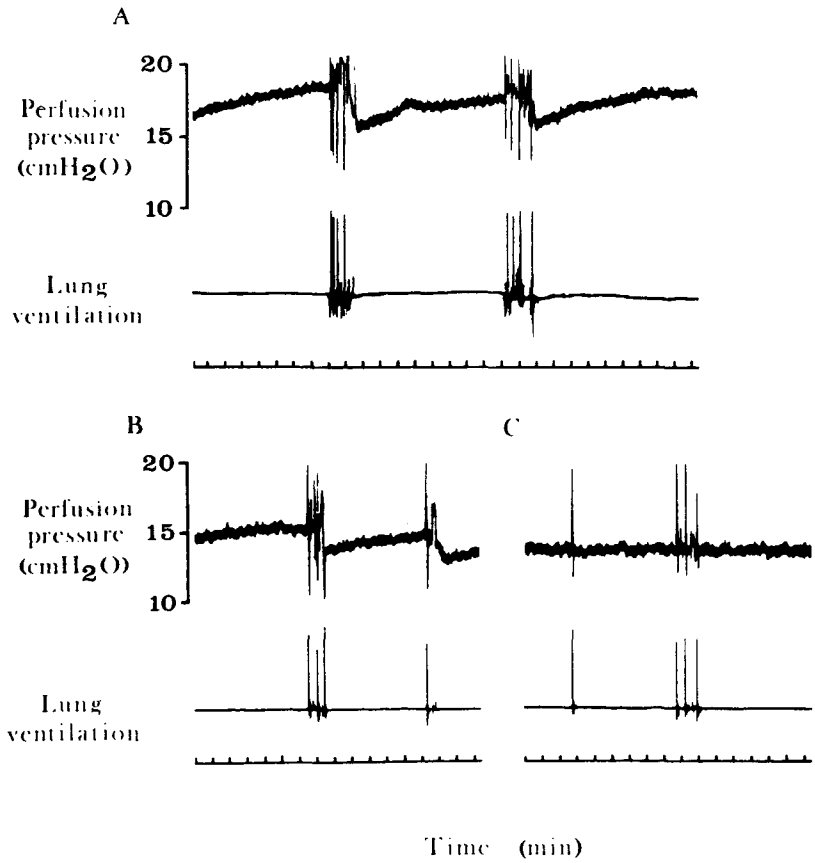

Fig. 8A–C. Changes in perfusion pressure of the entire perfused pulmonary circulation of restrained, lightly anaesthetized *Testudo graeca* **A** during voluntary apnoea and lung ventilation, **B** immediately before perfusion with 10^{-6} M atropine and **C** 5 min after atropine perfusion. Lung ventilation is indicated by vertical deflections in the ventilation record. Large perfusion pressure transients coincidental with lung inspiration are purely artifactual and occur because of the inverted posture of the animal. Note the progressive increase in perfusion pressure during apnoea in the non-atropinized animal

turtles and tortoises of 10–20 cm H_2O, which is at the bottom of their normal pulmonary blood pressure range (Shelton and Burggren, 1976). The perfusion pressure of the pulmonary circulation of both species showed a significant and relatively consistent increase as apnoea progressed (Fig. 8A). Perfusion pressure began to decline with onset of lung ventilation and had reached a minimal level within 30 s of the final respiratory movement, whereupon it began to increase once again. These changes in pulmonary resistance were cholinergically mediated, for perfusion with saline containing 10^{-6} M atropine completely eliminated all changes in perfusion pressure of the pulmonary circulation, apart from those pressure artifacts attributable to expiratory and inspiratory movements (Fig. 8B and C). Pulmonary perfusion pressures in atropinized animals usually stabilized at those levels which were evident immediately after lung ventilation before treatment with atropine.

In some experiments one of the distal pulmonary arteries was carefully severed at its point of entry into the lung and perfused in situ in the same

manner as was the entire pulmonary circulation. The distal pulmonary artery exhibited marked vasomotor responses during lung ventilation which closely resembled those observed during perfusion of the entire pulmonary circulation.

Discussion

A marked structural and functional heterogeneity has been revealed within the pulmonary arterial tree of *Pseudemys* and *Testudo*. The arterial wall of both the common pulmonary and proximal extrinsic arteries consists largely of elastic fibres with little vascular smooth muscle, and, as a consequence, is very distensible. Together these arteries constitute a highly compliant, centrally located volume reservoir within the pulmonary arterial tree. The distal pulmonary arteries share few of the properties of the more central vasculature. Only one-half as distensible and of much smaller diameter than the proximal vasculature, the distal vasculature forms a highly muscular peripheral conduit of low volume which leads directly into the pulmonary vascular bed. This structural arrangement within the pulmonary arterial tree has a profound effect on the dynamics of pulmonary blood flow. During the early phases of ventricular ejection arterial pressure increases rapidly in the highly elastic pulmonary reservoir, and as a result its volume increases substantially. Assuming a pulmonary stroke volume of 1.6 ml (Shelton and Burggren, 1976), an absolute volume distensibility for the extrinsic arterial pulmonary vasculature of 32.5 μl/cm H_2O (Table 1) and a blood pulse pressure of 20 cm H_2O, then at peak systolic pressure the pulmonary arterial tree of *Pseudemys* has been distended above its end diastolic volume by a volume equivalent to 40% of pulmonary stroke volume. When the pulmonary valves close at the end of systole, some 20% of the pulmonary stroke volume is still accommodated in the extrinsic arterial pulmonary vasculature. This volume, approximately 0.3 ml or 10% of the total stroke volume of the heart, flows through the distal pulmonary artery impedance into the pulmonary vascular bed strictly during diastole as the elastic reservoir passively recoils and imparts energy back into the circulation. This can be contrasted with the nature of blood flow in the central systemic circulation, where all blood flow occurs between the opening and closing of the systemic valves (White and Ross, 1966; Shelton and Burggren, 1976). Significant pulmonary blood flow maintained throughout diastole appears to be characteristic of the chelonian pulmonary circulation (White and Ross, 1966; Johansen et al., 1970; Shelton and Burggren, 1976), although the magnitude of diastolic blood flow will depend on the duration of the cardiac cycle and on the impedance at points peripheral to the output of the elastic reservoir. The pulse-damping effect on blood flow imposed by the central pulmonary reservoir is especially appropriate in the turtle and tortoise, which normally exhibit relatively low heart rates of only 6–20 beats/min (Burggren, 1975). Such prolonged cardiac cycles compared to many other vertebrates of similar size would result in a much greater flow pulsatility in the pulmonary arterial tree if the central pulmonary reservoir did not provide a proportionately long period of elastic recoil during diastole.

Manifestations of peripheral wave reflection, such as oscillations in impedance with increasing harmonic frequency, have in no instance been revealed in the pulmonary arterial circulation of *Pseudemys* and *Testudo*. This is not surprising, for wave reflection effects, maximal when the transit time between the heart and the peripheral reflecting sites is approximately 25% of the cardiac cycle, are generally insignificant when transit time is less than about 5% of the cardiac cycle (Langille and Jones, 1975). In *Pseudemys* the transit time between the heart and peripheral end of the distal artery is less than approximately 30 ms, compared with a cardiac cycle over a normal heart rate range of approximately 2.3–7 s. Wave transmission models which have been developed to explain haemodynamic anomalies in mammalian circulation resulting from peripheral wave reflection are thus apparently unnecessarily complex to apply to the chelonian pulmonary circulation. A much simpler 'lumped parameter' electrical analogue of the circulation, derived from the now-classic 'Windkessel' reservoir first proposed by Frank (1899), might be useful in describing the haemodynamics of the chelonian pulmonary circulation. This model describes a relatively simple arrangement in which blood from a central elastic reservoir which has been fully distended during systole flows out during the diastolic period through a single peripheral resistance; in terms of an electrical model, a capacitor which discharges with a time constant, t, through a single resistor. It is imperative, however, that blood pressure and blood flow change simultaneously at all points throughout such a 'Windkessel' reservoir. At the recording speeds of Figure 3, pressure transients in the chelonian pulmonary circulation such as the pressure wave 'foot' occur virtually simultaneously at the central end of the common pulmonary artery and at the peripheral end of the proximal extrinsic artery. In fact, a transit time through the pulmonary reservoir of only 20–25 ms can be indirectly determined from the present in vitro measurements of arterial dimensions and distensibility (see Shelton and Jones, 1968 for details of calculations). Therefore, in spite of a relatively low pulse wave velocity, the chelonian pulmonary 'Windkessel' is so short in length that pressure (and presumably flow) transients will occur virtually instantaneously throughout it, thus satisfying an important requirement of a lumped resistance-capacitance model.

How well, then, can blood pressure and flow relationships in the chelonian circulation be predicted from the 'Windkessel' model? In Figure 9 is presented a graph of the dependence upon harmonic frequency of input impedance modulus and phase as measured in the pulmonary artery of a *Pseudemys* during lung ventilation. Also presented are pulmonary impedances predicted by a 2 component (R-C) lumped parameter model (see Langille and Jones, in press, for details of arterial impedance calculations based on the 'Windkessel' model). The prediction of impedance modulus by this model is quite accurate at low frequencies, tending to slightly smaller values than measured at the highest frequencies. Impedance phase is not as well predicted as modulus by this model, since at all frequencies a larger phase lag is predicted than is actually measured. Increasing the complexity of the model by adding an inductance representing the considerable inertia of blood in the pulmonary arterial tree would tend to increase impedance modulus and decrease impedance phase, according to basic AC circuit theory. The simplest R-C model derived from the 'Windkessel'

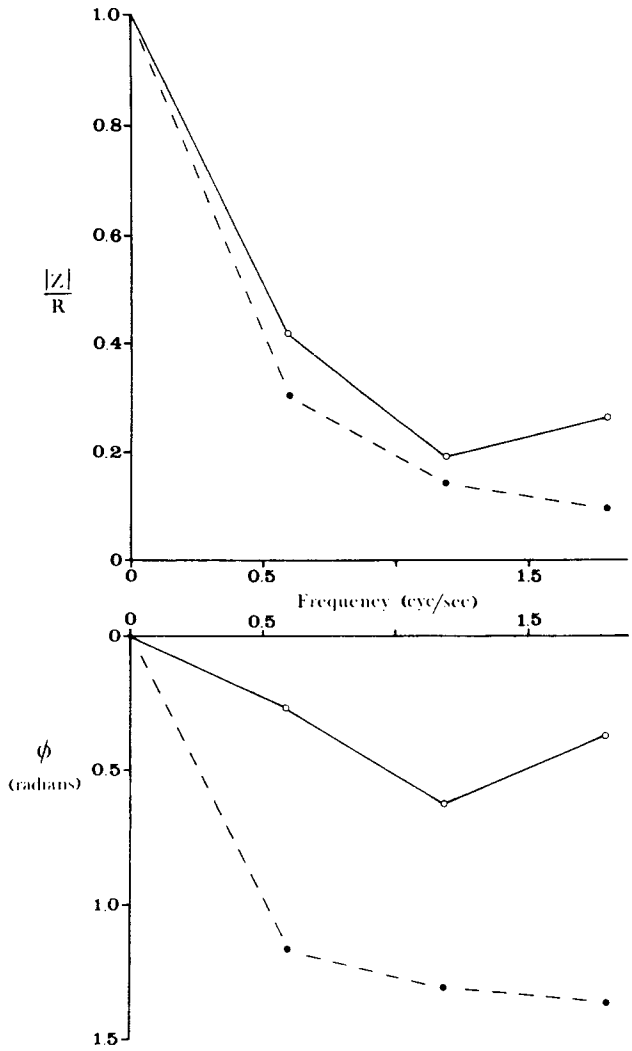

Fig. 9. Impedance modulus and phase computed for the pulmonary arterial circulation of a lightly anaesthetized *Pseudemys scripta* during voluntary breathing (solid lines). Values of impedance modulus and phase calculated from a lumped parameter resistance-compliance model are indicated by dashed lines

thus clearly has limitations in describing the dynamics of blood flow in the chelonian circulation, but it is nonetheless instructive to interpret in vivo changes in pulmonary arterial impedance and blood flow in such terms of localized (or lumped) regions of resistance and compliance. Now, the rate of blood flow from the pulmonary 'Windkessel' will be determined by a time constant which is the product of the distensibility of the central elastic reservoir and the resistance of the arterial vasculature beyond its peripheral output. The distensibility of the common pulmonary and proximal arteries, which constitute the pulmonary 'Windkessel' and account for some 90% of the absolute volume distensibility of the extrinsic pulmonary arterial vasculature (Table 1), could not be significantly altered in vitro by acetylcholine or adrenaline in any dosage. If the distensibility of the pulmonary 'Windkessel' is also effectively static in vivo, then variations in the time constant of the elastic reservoir must be

mediated solely by alterations in peripheral resistance produced by vasomotor activity of the extrinsic pulmonary arteries and the vasculature in the lung parenchyma. Cholinergically mediated changes in resistance beyond the peripheral output of the perfused pulmonary 'Windkessel' during intermittent lung ventilation in *Testudo* and *Pseudemys* are of considerable magnitude (e.g. increases of up to 5%/min during apnoea). Such changes in peripheral resistance are of the magnitude required to account for both the changes in pulmonary blood flow profiles and for the pulmonary minute flow variations which have been attributed to pulmonary vasomotor activity in unrestrained, intact Chelonia (White and Ross, 1966; Johansen et al., 1970; Shelton and Burggren, 1976).

Although located proximally to the elastic reservoir of the common pulmonary arteries, the impedance of the pulmonary outflow tract resides morphologically in series with the other elements of pulmonary impedance affecting flow from the 'Windkessel'- the distal pulmonary arteries and the pulmonary vascular bed. Vasomotor activity of the smooth muscle lining the pulmonary outflow tract will to some extent alter the balance between total pulmonary and systemic impedance and so ultimately redistribute cardiac output, although its contribution in the intact animal remains obscure. Earlier workers, relying on either direct observation or on pressures recorded from decerebrate, hemorrhaged preparations (Woodbury and Robertson, 1942; March, 1961), suggested that a large increase in outflow tract impedance could develop during apnoea. Blood pressure measurements on *Pseudemys* and *Testudo* (Shelton and Burggren, 1976) do not confirm any changes in impedance of the pulmonary outflow tract. This is surprising, for the present investigation has clearly demonstrated the potential for marked vasomotor responses in the perfused pulmonary outflow tract or in the intact animal during vagal stimulation. It is noteworthy that a large arterial impedance over the pulmonary outflow tract of chronically cannulated garter snakes has also been described (Burggren, in preparation), and it too is apparently static in vivo during normal intermittent breathing. It may transpire that vasomotor responses of the outflow tract of turtles and snakes only accompany prolonged periods of apnoea or activity, neither of which have normally occurred during these previously reported chronic pressure measurements.

Clearly, then, there are several potential sites throughout the chelonian pulmonary circulation where variation of single or multiple combinations of parameters affecting pulmonary blood flow can occur. Parasympathetic vagal stimulation produced a uniformly excitatory effect, i.e. vasoconstriction, in the pulmonary vascular bed, the extrinsic pulmonary arteries and the pulmonary outflow tract. Stimulation of sympathetic fibres mediating chronotropic and inotropic cardiac responses never resulted in a vasomotor effect in either the pulmonary outflow tract or distal pulmonary artery of *Pseudemys* and *Testudo*. Yet, perfusion of the cholinergically excited outflow tract or distal pulmonary vasculature of *Testudo* and *Pseudemys* with adrenaline produced a marked vasodilation in the present investigation (Fig. 6B and 7B). Perhaps in the intact animal a rapid increase in the concentration of circulating catecholamines and a rapid decrease of parasympathetic neural activity act together to produce the abrupt fall in pulmonary impedance concomitant with the onset of lung ventilation.

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