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CARDIOVASCULAR DYNAMICS OF THE CHELONIA DURING APNOEA AND LUNG VENTILATION

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SUMMARY

1. In both *Pseudemys* and *Testudo*, blood pressures are identical in all chambers of the ventricle. Systolic pressures are not measurably different in the ventricle and systemic arteries but are 0·5–2·0 cm H_2O lower in the pulmonary artery due to the resistance of the pulmonary outflow tract. Diastolic pressures are the same in all systemic arteries but are substantially lower in the pulmonary artery. It is concluded that the systemic and pulmonary circulations are perfused by a single pump during both apnoea and lung ventilation.

2. Flow profiles in pulmonary and systemic arteries are characteristically different. Substantial changes in cardiac output may occur during intermittent breathing and apnoea, especially if there are large fluctuations in heart rate. Pulmonary flow increases during lung ventilation due to vasodilation of the lung vasculature. Systemic flow is also affected though the increase is usually smaller.

3. Any separation in the blood pumped by the single ventricle must be maintained by laminar flow patterns and the composition of the blood in each of the major arteries should reflect their relationship to these patterns.

INTRODUCTION

Because they show a number of anatomical peculiarities representing putative stages in the evolution of a completely separate systemic and pulmonary circulation, present-day reptiles are of considerable interest to cardiovascular physiologists. In crocodilians the ventricular division is complete but the left aortic arch emerges with the pulmonary artery from the right ventricle. White (1968, 1969, 1970) has suggested that this arrangement is not as unsatisfactory as may seem to be the case, because the valves between right ventricle and left aorta seldom open, at least when the animal is ventilating its lungs. During a dive the pressures within the right ventricle rise sufficiently to cause the valves to open and some right-to-left shunt of blood then occurs. The potential for separation of pulmonary and systemic blood is even further reduced in the squamate and chelonian reptiles because they have an incomplete ventricular septum. In spite of this, a variable degree of blood separation does occur in most of the animals so far examined (Steggerda & Essex, 1957; Millen *et al.* 1964; Tucker, 1966; White, 1970). Even amongst these groups there are considerable differences in heart function and in the relationship between systemic and pulmonary

circulations. For example, Millard & Johansen (1974) have suggested that a much greater degree of independence exists between right and left sides of the heart in varanid lizards than seems to be the case in other squamate or chelonian reptiles. It seems likely that such differences may relate as much to environmental relationships as to phylogenetic position. In the present study, cardiovascular function was examined in two chelonians of very different habit.

Early investigations of the chelonian circulation by Shannon & Wiggers (1939) and Woodbury & Robertson (1942) ably demonstrated many general properties of the system, but the limitations of their manometric systems and the lack of blood flow measurements precluded extensive analysis. The application of modern techniques of pressure recording by Steggerda & Essex (1957) led them to the view that, though there were no significant differences in pressures within the dorsal ventricular chambers (cavum arteriosum and cavum venosum – see Fig. 1) of the turtle *Chelydra*, some separation did develop during systole between these chambers and the cavum pulmonale. Certainly, dye dilution methods demonstrated that a considerable separation of systemic and pulmonary venous blood was maintained. Other workers (see White, 1968) also concluded that the rearrangement during systole of muscular ridges within the ventricle was capable of producing functionally distinct pumps under some conditions.

In addition to possible ventricular division, the importance of changes in resistance of pulmonary and systemic vasculature in determining the distributional fate of blood within the chelonian ventricle has also been emphasized, though differences of interpretation exist. Resistance changes may occur in the peripheral vasculature (White, 1968; Johansen, Lenfant & Hanson, 1970), in the extrinsic arteries leading to the lungs (Berger, 1972), or in the pulmonary outflow tract itself (Woodbury & Robertson, 1942; March, 1961). White & Ross (1965, 1966) measured both pressures and flows in the arterial arches of the turtle, *Pseudemys*, during enforced and non-enforced dives. They reported that during diving, as pulmonary flow decreased and a right-to-left blood shunt developed in the heart, systolic pressures in the pulmonary and systemic circulations were identical. During lung ventilation, however, flow relationships were markedly different as a left-to-right shunt prevailed and pressures in the pulmonary arteries became lower than those in the systemic vessels throughout the entire cardiac cycle. Johansen *et al.* (1970) also reported a similar lack of coincidence in the pulmonary and systemic pressures measured in *Pseudemys*. White (1968) attributed these changes during breathing to both pulmonary vasodilation and ventricular separation. His work did not eliminate the possibility that the properties of the pulmonary arteries and the outflow tract were also of significance.

Clearly, there are several mechanisms, both within and peripheral to the chelonian heart, which previous investigations suggest may affect the fate of blood entering the ventricle from right and left auricles. Moreover, the intermittent pattern of chelonian lung ventilation and the accompanying fluctuations in many physiological parameters may produce marked shifts between the mechanisms or sets of mechanisms that control arterial blood flow. This investigation was undertaken to examine the pressure and flow relationships in the heart and major arteries of two chelonian species, and to determine what modifications of these relationships might accompany lung ventilation, apnoea and other physiological events.

METHODS

The animals used in this study were the Greek tortoise, *Testudo graeca* (L.), and the red-eared turtle, *Pseudemys scripta* (Schoepff). They weighed between 1 and 1·5 kg and were obtained from commercial sources. All animals were kept in the laboratory at 20–22 °C and were apparently healthy at the time of experimentation.

Chronic and acute experiments were carried out, and in both cases the animals were initially anaesthetized for the operations to implant pressure catheters and flow probes. Anaesthetic injection, using 12–20 mg pentobarbital (Nembutal)/kg body weight, and inhalation, using halothane vapour in a closed chamber (Gans & Hughes, 1967), were both tried. Inhalation methods are difficult to control (Kaplan, 1969) and halothane was only satisfactory in those experiments in which the animals could be artificially ventilated to produce recovery. This was not possible in the majority of cases. Intraperitoneal injection of Nembutal (Hunt, 1964; Kaplan, 1969) induced variable levels of anaesthesia, even when used in one animal on different occasions. Thus, chemical anaesthetics were not very satisfactory, particularly in acute experiments, which required a quick and reliable recovery after a fairly brief period of surgery. The most suitable method, which was used in the majority of experiments, was to induce a cold torpor (White & Ross, 1966) by placing the animals in a 1 °C cold room for 12–15 h before surgery. Recovery was easily controlled by allowing the animals to warm to room temperature.

During surgery the anaesthetized animal was held ventral side up by means of cords passed around the legs. A hole 4 cm square, directly over the heart and major arteries, was cut in the plastron with a small circular saw mounted in a dentist's drill. The saw blade made a very fine cut so that the excised piece of plastron would fit back into the hole with a very little clearance. The common pulmonary artery, left and right aorta, and, in some experiments, the brachiocephalic artery were non-occlusively cannulated in an upstream direction with PP25 (0·4 mm bore, 0·8 mm diam.) polythene tubing. A section of the relevant artery was closed off by means of small artery clamps, a hole was made in the arterial wall with a 26-gauge hypodermic needle, and the cannula was inserted through the hole. In most experiments it was anchored in position with a loop of surgical silk. Blood loss was invariably negligible, and after recovery from anaesthesia the central cardiovascular system appeared to be functioning normally in every respect in all experiments described below.

In some acute experiments the ventricular chambers were also cannulated. This made it necessary to open the pericardium, though such disruption was kept to a minimum. A needle was passed through the ventral myocardium into one of the three ventricular chambers, the cavum pulmonale, cavum venosum or cavum arteriosum (see Fig. 1). The needle was withdrawn and the tip of a PP60 (0·8 mm bore, 1·2 mm diam.) polythene cannula was advanced through the hole into the underlying ventricular chamber. The cannula was held in place by silk stitches in the superficial layers of the ventricle wall. One, two or three of the ventricular chambers were cannulated in this way and the position of the cannula tips was confirmed by dissection at the end of each experiment. All cannulae were filled with heparinized saline, and 200 i.u./kg body weight of heparin were injected into the animal at the beginning of each experiment.

The blood pressures in the arteries were measured with Sanborn 267B transducers. When these manometers were connected to cannulae of PP25 tubing 25–35 cm long the undamped natural frequency of the whole recording system was 45 c/s, with a damping value 37% of critical. Bio-Tech BT 70 transducers were used to measure intracardiac pressures and these, with PP60 cannulae 20–25 cm long, produced an overall, undamped natural frequency of 69 c/s with 15% critical damping. The heart rate was some 0·2–0·5 c/s and so it was assumed that both pressure recording systems were adequate to record even the fastest transients without significant amplitude or phase lag error. Consequently, no corrections were applied to the data during analysis. The total pressure recording system was tested for frequency response and damping characteristics at the beginning of every experiment, before the cannulae were surgically implanted. Pressure calibrations and zero levels could be applied throughout an experiment and frequent checks were made on the manometers to eliminate drift and sensitivity changes.

Blood-flow measurements were made in all the major arteries emanating from the heart, though the left pulmonary artery and the right aorta were the vessels usually examined. A Biotronix BL410 electromagnetic blood flow meter was used in the determinations. The flow probes, whose lumen sizes varied from 1·0 to 3·0 mm diam., were positioned on the arteries distal to the pressure cannulae. In acute experiments, zero flow was determined by mechanically occluding the blood vessel. In chronic experiments, zero flow in the systemic vessels was apparent from inspection of the trace. Zero flow in the pulmonary artery was more difficult to determine but a constant level of trace immediately before systole, particularly when heart rates were low, was assumed to give a reliable zero. *In vitro* calibration of the flow probes was carried out at the end of every experiment, using saline delivered from a reservoir set at a height equivalent to the animal's mean blood pressure. The saline was allowed to flow through pieces of artery excised from the experimental animal and calibration readings were taken from the flow probe through which the artery passed.

Blood flows and pressures were recorded directly on to heat-sensitive paper using a six-channel Sanborn 966 series recorder, writing on rectangular co-ordinates. Other parameters such as heart rate, stroke flow and minute flow were extracted from the recorded data. Stroke flow was determined by integration of the flow curves using weighing or square counting methods.

After cannula and flow probe implantation, the animals were allowed to warm to room temperature (18–20 °C). For the duration of acute experiments, which was some 5–10 h, the animals were restrained ventral side up. In the chronic experiments, which lasted up to 4 days, the pressure cannulae and flow probe leads were passed through small apertures cut into the excised square of plastron, which was subsequently fastened back into position with a rapidly setting epoxy resin. The animal was then turned upright and placed in a small holding tank to recover. The tank was dry in experiments on tortoises and contained water to a depth of 10–15 cm when turtles were used. Some difficulty was experienced in positioning the probes and cannulae so that they would continue to work satisfactorily in the chronic preparation. Fortunately the resin fixing the plastron square was easily removable so that repositioning was possible when initial implantations failed. When the animals had recovered they were allowed to move freely within the restricted space offered by the

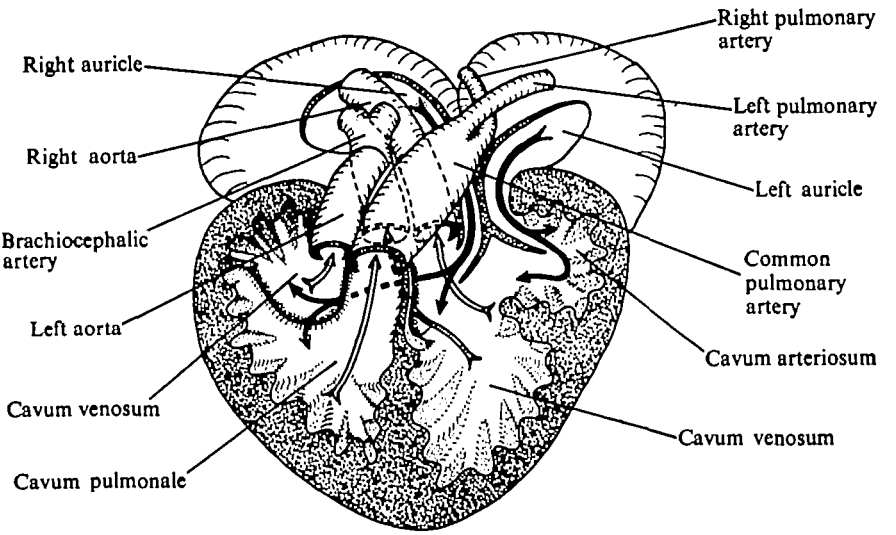

Fig. 1. Diagrammatic illustration of the chelonian heart. The heart is shown in a ventral aspect. The cavum pulmonale, from which arises the common pulmonary artery, lies ventral to the cavum venosum. All of the systemic arteries arise from the cavum venosum. The solid arrows indicate the gross movement of blood from the auricle into the incompletely divided ventricle. The open arrows indicate movement of blood from the ventricular chambers into the arterial arches. Arrows are not intended to illustrate the flow of separate blood streams through the ventricle.

tank and, in the case of *Pseudemys*, to dive and surface voluntarily. The measurements were then taken from these unrestrained animals.

RESULTS

In most respects the haemodynamics of the central arterial circulations in *Pseudemys* and *Testudo* are very similar. A general description applicable to both animals will be given, and such qualitative or quantitative differences in pressures and flows as do exist will be made apparent. The data on basic pressure and flow relationships were taken from animals breathing normally in air. The section on pressures deals, in the main, with experiments performed on acute preparations, that on flows with experiments on chronic preparations. A final section describes the modifications to pressure and flow seen in chronic preparations as a result of apnoea, diving and general activity.

1. *Gross anatomy*

Physiological interest in the chelonian heart stems largely from the anatomical relationships between the undivided ventricle, the two auricles, and a number of arterial arches which always include separate left and right aortae. Chambers within the single ventricle are more or less clearly demarcated by ridges in the myocardium. The right auricle opens into one such chamber (the cavum venosum) and the left auricle into another (the cavum arteriosum) (Fig. 1). The arterial outlets from the ventricle emerge both from the front of the cavum venosum and from a third chamber, the cavum pulmonale. In the chelonians the right aorta and brachiocephalic

Table 1. *Arterial blood pressures in the turtle* Pseudemys scripta *and the tortoise* Testudo graeca

(Values presented are means ± 1 standard error in cmH_2O. Blood pressure for each turtle or tortoise was calculated from pooled measurements from both acute and chronic recordings. A mean value for both species was then calculated from these data.)

Species	Pulmonary systolic pressure	Pulmonary diastolic pressure	Systemic systolic pressure	Systemic diastolic pressure	*N*
Pseudemys scripta	33·7 ± 1·8	13·1 ± 1·1	34·3 ± 1·7	25·0 ± 1·4	15
Testuda graeca	46·7 ± 2·0	16·4 ± 1·6	47·4 ± 2·1	30·6 ± 1·8	15

artery are more closely associated with the cavum venosum, the pulmonary artery with the cavum pulmonale and the left aorta occupies a somewhat intermediate position. The cavum arteriosum has no direct connexion with the arterial arches, and clearly for the heart to function there must be a considerable amount of blood transfer between the different ventricular chambers. The anatomical relationships of the chelonian heart have been more fully described by Mathur (1946) and Khalil & Zaki (1964).

2. *Ventricular and arterial blood pressures*

Both systolic and diastolic pressure were significantly higher in *Testudo* than in *Pseudemys*. Average values are given in Table 1. Fig. 2 shows representative pressures in the heart and arterial arches of *Pseudemys* and *Testudo* as recorded in acute experiments. Pressure waveforms recorded from the cavum pulmonale, cavum venosum and cavum arteriosum were all superimposable, throughout the entire cardiac cycle. Auricular contraction usually made a late contribution to ventricular filling and increased intraventricular pressure by 1 or 2 cmH_2O. The ventricular myocardium then began to contract, causing a brief initial phase of isometric activity with pressure rising simultaneously in all parts of the ventricle. The rate of pressure rise was somewhat variable, being greatest during periods of lung ventilation, as in Fig. 2, and falling appreciably during extended periods of apnoea. Because diastolic pressures in the pulmonary circulation were substantially lower than those in the systemic (Table 1), the valves between the cavum pulmonale and the pulmonary artery opened and pulmonary ejection began before blood was ejected into the systemic vessels (Figs. 2, 3A). The rate of intraventricular pressure rise was little affected by pulmonary outflow and, some 60–100 ms afterwards, the pressure in the systemic vessels was exceeded and systemic ejection initiated. Pressures in all parts of the ventricular–central arterial system were, at this time, virtually the same, the small gradients involved in blood flow not being resolvable with the apparatus used. In many turtles and some of the tortoises (Figs. 2B, C) the high rate of pressure rise continued for a very short time after the start of systemic ejection, but as systemic flow became established an inflexion was apparent in all arteries after which pressure increased more slowly to a peak. In other animals this change in slope of the pressure waveform occurred as the systemic valves opened. Pressures began to fall simultaneously in all parts of the central circulation. Closure of the sets of valves between the ventricle and all three systemic vessels took place synchronously and was usually marked by

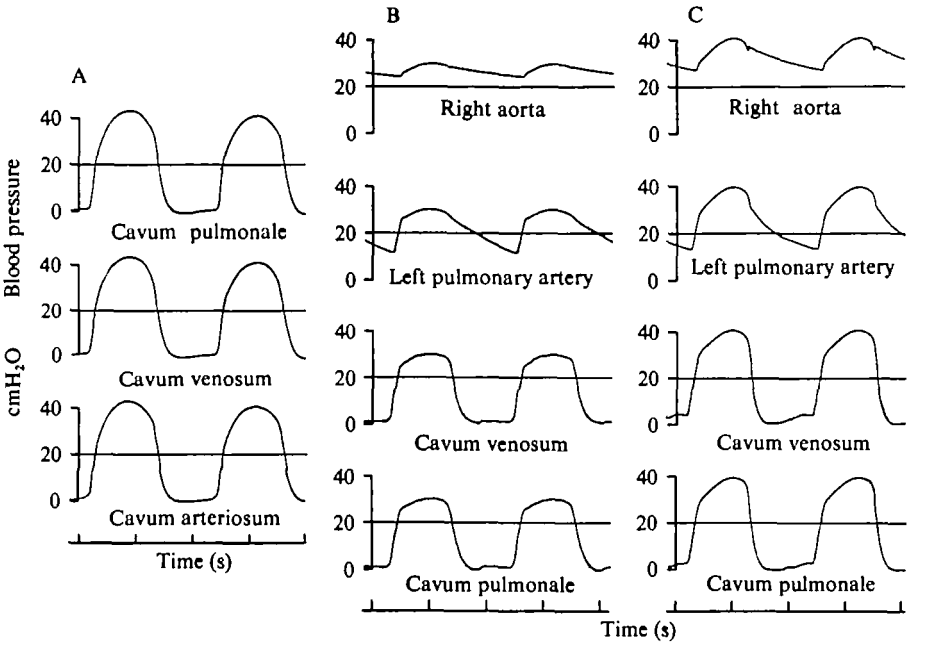

Fig. 2. Simultaneously recorded intraventricular and arterial pressures. (A) Pressure in the cavum arteriosum, cavum venosum, and cavum pulmonale of a restrained unanaesthetized *Testudo*. Intraventricular and arterial pressures in (B) restrained *Pseudemys* and (C) restrained *Testudo*.

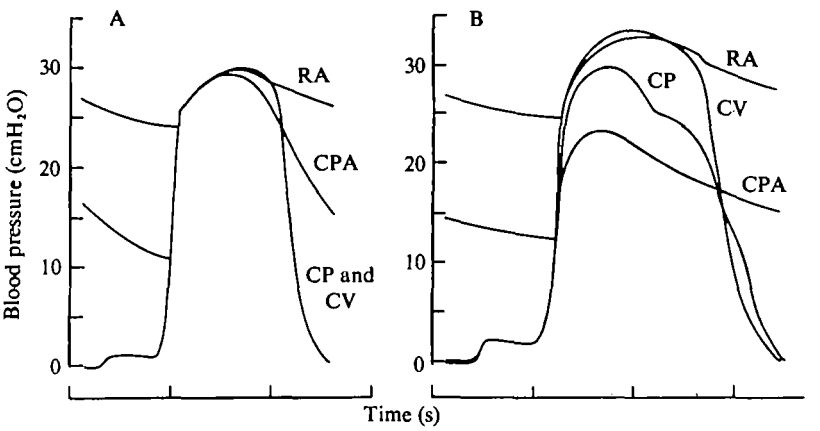

Fig. 3. Superimposed blood pressures during a single cardiac cycle recorded from the cavum pulmonale (CP), cavum venosum (CV), common pulmonary artery (CPA) and right aorta (RA) of (A) *Pseudemys*, and (B) *Testudo*. In the case of the tortoise the animal was heavily sedated with Nembutal and was being artificially ventilated.

a notch in the arterial pressure traces. The timing of pulmonary valve closure was more difficult to determine because the recordings were made in or peripheral to the large elastic reservoir of the common pulmonary artery and no valve notch was seen. However, when the cannula was positioned close to the pulmonary valves (Fig. 4), a notch in the pulmonary pressure trace occurred some 100 ms later than that in the systemic vessels.

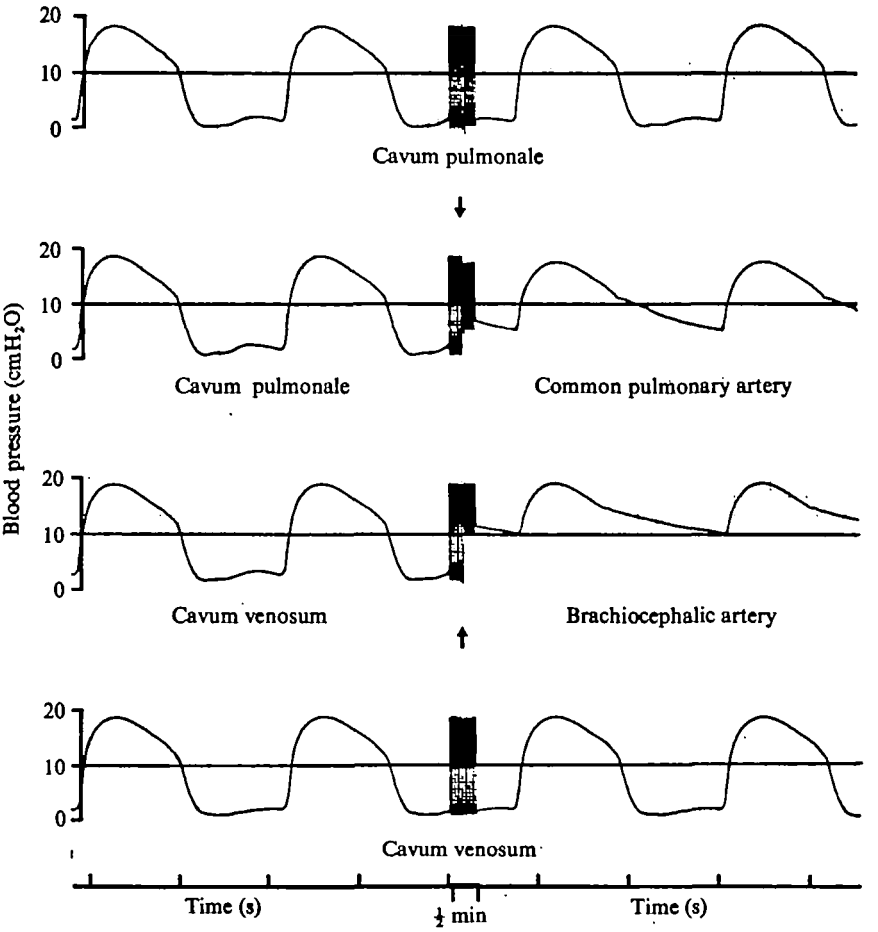

Fig. 4. The effect of withdrawing cannulae tips from the ventricular chambers back into the arterial circulation in *Pseudemys*. At the arrows (slow time base) the cannulae introduced into the cavum pulmonale and cavum venosum were simultaneously withdrawn through the valves into the common pulmonary and brachiocephalic arteries, respectively.

Pressure waveforms recorded from the left aorta, right aorta and brachiocephalic arteries were invariably identical (see Fig. 7) and between the opening and closing of the systemic valves were superimposable on the ventricular pressure waveform (Fig. 3A). Pressure run-off in the pulmonary circulation during diastole was more rapid than in the systemic vessels, resulting in an average diastolic pressure difference between the two circulations of 12 cmH_2O in *Pseudemys* and 15 cmH_2O in *Testudo*. During the early stages of systole, pressure in the common pulmonary artery was identical to or less than 1 cmH_2O lower than pressures in the ventricle and other arteries. However, as systole continued a slightly larger pressure difference of 0·5–3·0 cmH_2O progressively developed between the ventricle and the common pulmonary artery in 12 of the 16 turtles and tortoises subjected to cardiac cannulation. These small pressure differences, although approaching the limits of resolution of the instruments, were significant at the 0·005 level.

Since the pressure gradient was small during early ejection, when the pulmonary

flow might be expected to be high, but increased later when flow was falling, it seemed likely that the pulmonary outflow tract was presenting a small but progressively increasing resistance to flow as each cardiac cycle developed. The resistance of the systemic outflow tracts, on the other hand, was so small as to give no detectable gradient at any time in the cardiac cycle. To examine this relationship further, acute experiments were performed on five animals in which the tips of the cannulae located in the arterial arches were carefully manoeuvred through the aortic valves into the cavum pulmonale or cavum venosum. The results of an experiment of this type are shown in Fig. 4. When the cannulae in the brachiocephalic and common pulmonary arteries were advanced into the cavum venosum and cavum pulmonale, respectively, identical pressure waveforms were recorded from these 'arterial' cannulae and those permanently implanted in the ventricular chambers. Simultaneous withdrawal of the 'arterial' cannulae back through the valves restored the original arterial pressure waveforms and confirmed the small drop in pressure over the pulmonary outflow tract. In the experiment shown in Fig. 4 the peak systolic pressure in the common pulmonary artery was approximately 1–2 cmH_2O less than that in the cavum pulmonale, whereas the brachiocephalic pressures were identical with those in the ventricle during the whole ejection phase. Interestingly, when the acute experiment was begun on this turtle, the pulmonary gradient was very small (< 1 cmH_2O) but briefly increased to some 2·5–3·0 cmH_2O during the first few attempts to manoeuvre the cannulae through the valves, and later fell back to the levels seen in Fig. 4. The very reactive nature of some parts of the pulmonary arterial vasculature and the constrictor effects of any sort of manipulation of the vessels was noticed on several occasions. It is possible that the act of implanting cannulae contributed to the production of the observed pressure gradients. However, the gradients were long lasting and persisted in chronic preparations at low but fairly constant levels.

This pattern of identical ventricular and arterial pressures, with detectable pressure gradients of very small size in the pulmonary outflow tract alone, was seen in all but one specimen of *Testudo* and one of *Pseudemys*. In these two animals the pressure waveforms in the cavum venosum and cavum pulmonale were not superimposable during ventricular contraction (Fig. 3 B). Additionally there was a large gradient of 4–7 cmH_2O over the pulmonary outflow tract, and as a consequence substantial differences in the systemic and pulmonary pressures occurred throughout the cardiac cycle. Waveforms of this type usually proved to be artefacts, due to air bubbles or blood clots in the cannulae or to occlusion of the cannula tip by contact against the myocardium. In the single *Testudo* showing the aberrant pressure waveforms these possibilities were eliminated. Most convincingly, the differences were confirmed by moving the pulmonary arterial cannula through the valves into the cavum pulmonale. In this particular acute experiment the animal was anaesthetized with Nembutal (15 mg/kg intraperitoneally) and was being artificially ventilated. The pressures in the single *Pseudemys* came from an unanaesthetized, chronic preparation but in this case extensive controls to eliminate artefact could not be undertaken.

3. *Arterial blood flow*

Almost all the experiments on blood flow were carried out on animals in which the flow probes and pressure cannulae were chronically implanted and the animals

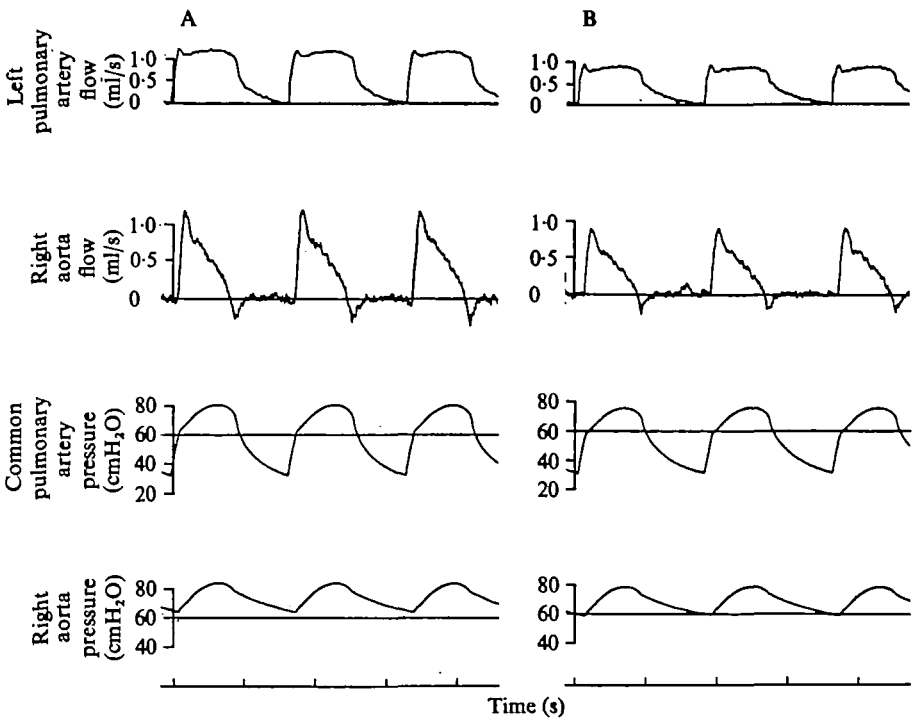

Fig. 5. Records of pulmonary and systemic blood pressures and blood flows in an unrestrained *Pseudemys* (A) during a breathing series, and (B) after 5 min of a voluntary dive.

were conscious and unrestrained. Comparison with acute preparations or with the chronic preparations during surgery to implant the probes revealed no significant differences in the pressure flow relationships between the two types of preparation that were not attributable to posture. Though most of the systemic flow records were obtained from the right aorta, control experiments demonstrated that the flow pulses in the left aorta, subclavian and carotid arteries were of similar profile.

Blood-flow waveforms in the systemic arteries of both *Pseudemys* (Fig. 5A) and *Testudo* (Fig. 6) were very different from those recorded in the pulmonary circulation. As described above, diastolic pressures were lower in the pulmonary artery, and thus flow started first in this vessel and had accelerated to maximum velocity by the time systemic flow was just beginning about 100 ms later. Often there was a sudden fall from peak flow in the pulmonary artery as systemic flow was established (Figs. 5 and 6, though more marked falls were seen in other cases). Thereafter, pulmonary flow fell only slowly until an inflexion and substantial decrease in flow occurred following pulmonary valve closure. In the systemic arteries, however, blood flow decreased at a relatively rapid and uniform rate following maximum velocity until a flow reversal occurred and the aortic valves closed. After valve closure there was no systemic flow until the beginning of the next ejection phase. Flow reversal responsible for closing the pulmonary valves was never seen at the pulmonary recording site which was distal to the large, centrally situated, elastic reservoir (Fig. 1). A substantial level of blood flow was maintained in the pulmonary arteries after valve closure (Figs. 5, 6).

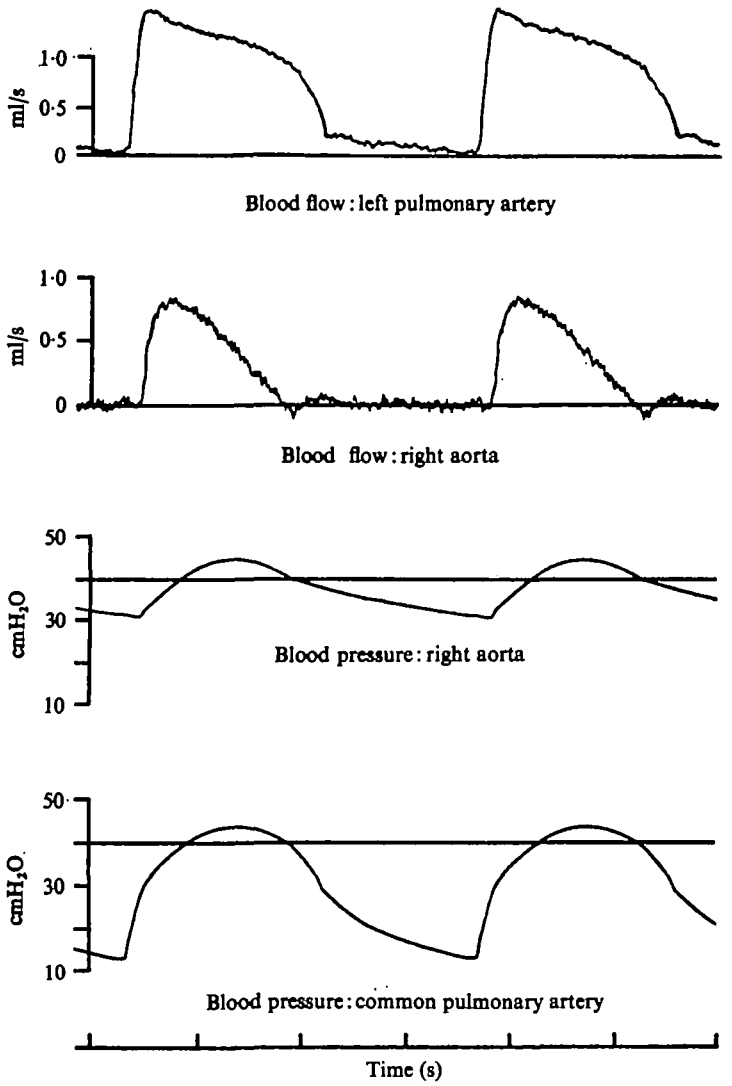

Fig. 6. Pulmonary and systemic blood pressures and flows in an unrestrained *Testudo*.

The differences between flow patterns in pulmonary and systemic vessels must reflect the different physical characteristics in the length and compliance of the two sets of tubes into which the ventricle is pumping, since the evidence suggests that the ventricle itself is acting as a single, undivided source. The systemic arterial system in these animals is made up of long vessels of relatively low compliance whereas the pulmonary arteries are short and highly compliant with a considerable part of the compliance situated central to the flow probe. The amphibian circulatory system possesses similar properties and the relationships have been discussed by Shelton (1975).

In actively ventilating turtles and tortoises, right aorta stroke flow was usually about 50% of that in the left pulmonary artery (Table 2). In one ventilating tortoise,

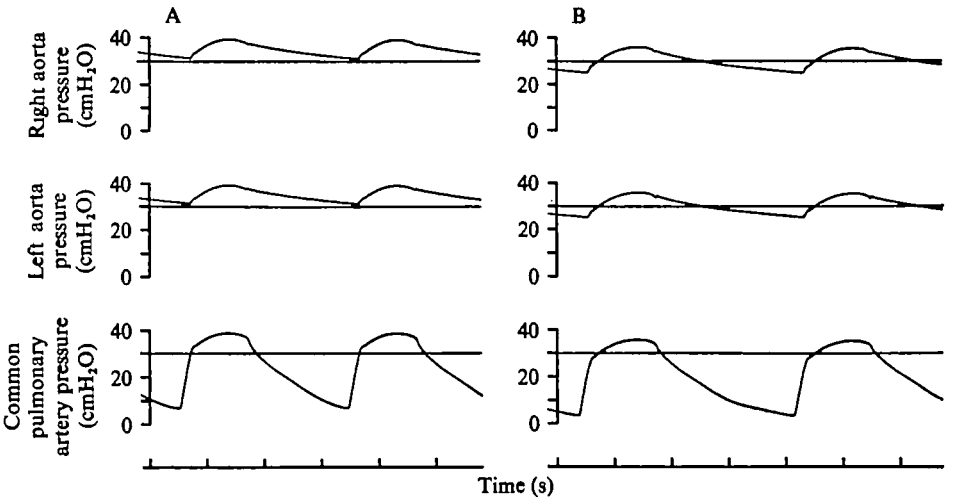

Fig. 7. Arterial pressures recorded in the left aorta, right aorta and common pulmonary artery of an unrestrained *Pseudemys* (A) during a breathing series, and (B) after 10 min of a voluntary dive.

extensive blood flow measurements allowed an estimation of total stroke flow in the arterial circulation to be made. Assuming equal flow in the left and right pulmonary arteries, total pulmonary stroke flow was approximately 1·84 ml. Total systemic stroke flow was approximately 1·60 ml, including the right aorta (0·58 ml), left aorta (0·46 ml), left and right subclavian arteries (0·48 ml) and left and right carotid arteries (0·08 ml), and thus total cardiac stroke output in this specimen was approximately 3·5 ml. Heart rate was 20 beats/min so total minute flow was 70 ml/min. Since these levels of left pulmonary and right aortic stroke flow were not substantially different from those mean values for both *Pseudemys* and *Testudo*, we have used the relationships from this one animal to derive the factors for calculating approximate cardiac outputs in Table 2.

4. *Factors affecting blood pressures and flow*

(i) *Lung ventilation*

The pressure measurements described in section 2 above, showing identical waveforms in all parts of the ventricle and similar systolic levels in ventricle and arterial arches, were sufficiently different from those in previous reports (White, 1968; Johansen *et al.* 1970) as to require exacting tests of their validity. The acute experiments were therefore followed by a series of 15 experiments in which two or three cannulae were chronically implanted in the arterial arches and the animals were allowed to recover. In these experiments the pericardium was left intact and the section of plastron replaced after minimal interference with the central arterial system. Animals of both species returned to the experimental tank appeared to be capable of surviving indefinitely.

The acute experiments were confirmed in that systolic pressures in all the systemic vessels were identical in value and in the pulmonary artery were approximately 1 cmH_2O lower, particularly towards the end of systole (Fig. 7A). Diastolic levels were also identical throughout the central systemic system, whereas they were

invariably lower in the pulmonary circulation. Although ventricular pressures were not measured in these experiments, the similarity between arterial pressures from acute and chronic preparations suggests that the experimental conditions have no effect on the ventricular–arterial relationships. During voluntary apnoea, which in *Pseudemys* could last up to 40 min, we found in every instance that there was no change in these basic pressure relationships. Though the rate of pressure rise, the overall profile of the pressure waveforms, the frequency of heart beat, and the absolute values for systolic and diastolic pressures were usually all altered during such a dive (Fig. 7B), the systolic levels were effectively identical and the pulmonary diastolic pressure was much lower than that recorded in all the systemic vessels. Similar results were also obtained from other chronic experiments in which, in order to implant flow probes, there was rather more surgical interference with the central arterial system. Even when there was a marked variation in heart rate associated with the change from apnoea to lung ventilation (especially in *Pseudemys*, where the fluctuations in heart rate were more marked than in *Testudo*; see Burggren, 1975), the pressure relationships described above were maintained (Fig. 8).

Though the general features of the pressure relationships were unaltered, changes in the absolute levels of blood pressure could sometimes be correlated with the ventilation–apnoea cycle. In those animals which showed substantial variation in heart rate, the tachycardia associated with lung ventilation was accompanied by somewhat elevated systolic pressures and decreased pulse pressures (Fig. 8). In other chronic preparations there was much less variation in heart rate, and in both *Testudo* (Fig. 9A) and *Pseudemys* (Fig. 9B), apart from small cyclical pressure fluctuations accompanying the ventilation movements, only very small changes in blood pressure were seen during the apnoea following ventilation. In some cases both systolic and diastolic pressures tended to decrease slightly during, and immediately after, periods of ventilation, especially if these were prolonged, and then increase again during the subsequent apnoea. However, other fluctuations of greater amplitude were often seen (Fig. 9A). These were not attributable to any obvious stimulus or activity and often obscured any small changes that may have been associated with ventilation.

Regardless of the effect on the pressure waveform, lung ventilation invariably caused an increase in pulmonary blood flow in both species. In *Pseudemys* showing ventilation tachycardia (Fig. 8), minute flow in the right aorta and the left pulmonary artery went up considerably as cardiac frequency, and, to a lesser extent, stroke flow increased, reflecting a large change in cardiac output (Table 2). Fluctuations in flow in those animals with a more uniform heart rate were less marked, though again considerable changes in pulmonary stroke flow could be seen when *Pseudemys* ventilated its lungs (Fig. 9B, Table 2). If several breathing series rapidly followed one another the effect on pulmonary stroke flow was cumulative. In *Testudo*, which exhibits much shorter periods of apnoea, the stroke flow changes were smaller (Fig. 9A, Table 2) though still quite evident, especially when the animal made a prolonged sequence of breathing movements. Isolated breaths, however, tended to have no effect on pulmonary blood flow. Because there was substantial variation in the arterial blood flows measured in different animals, the data relating to apnoea and lung ventilation in Table 2 were collected in pairs. Stroke flows were measured during a period of lung ventilation and during the immediately preceding or following

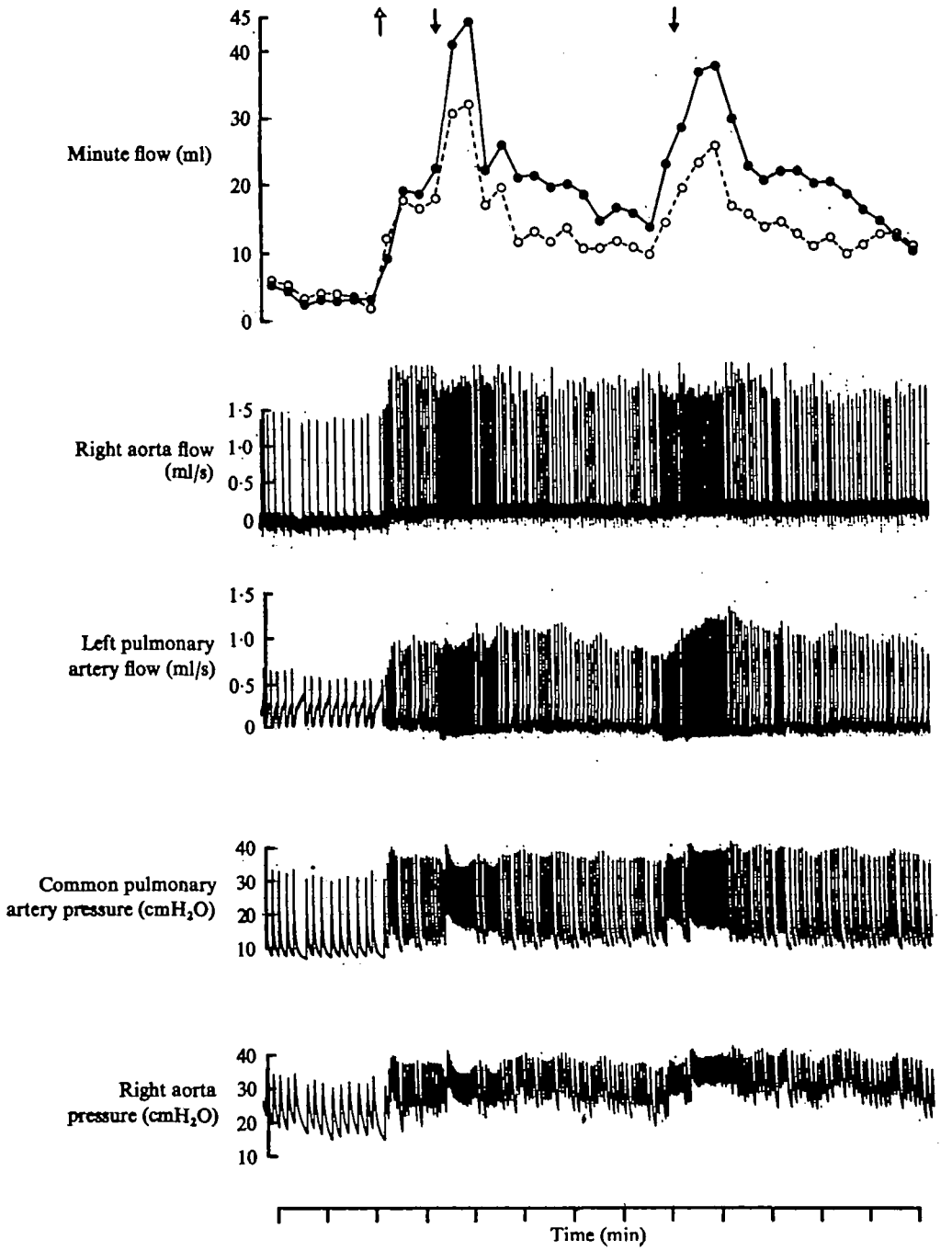

Fig. 8. Effect of lung ventilation and accompanying tachycardia on systemic and pulmonary blood pressure and flow in an unrestrained *Pseudemys*. At the start of the records the turtle was voluntarily diving. The turtle surfaced (first arrow), then began a short period of lung ventilation (second arrow). This was followed after 4 min by another short period of lung ventilation (third arrow). The flow records were used to derive minute flow as plotted in the graph. Closed circles indicate left pulmonary minute flow, open circles right aorta minute flow. (At very low heart rates the pulmonary flow probe shows some zero drift due to reduced vessel size.)

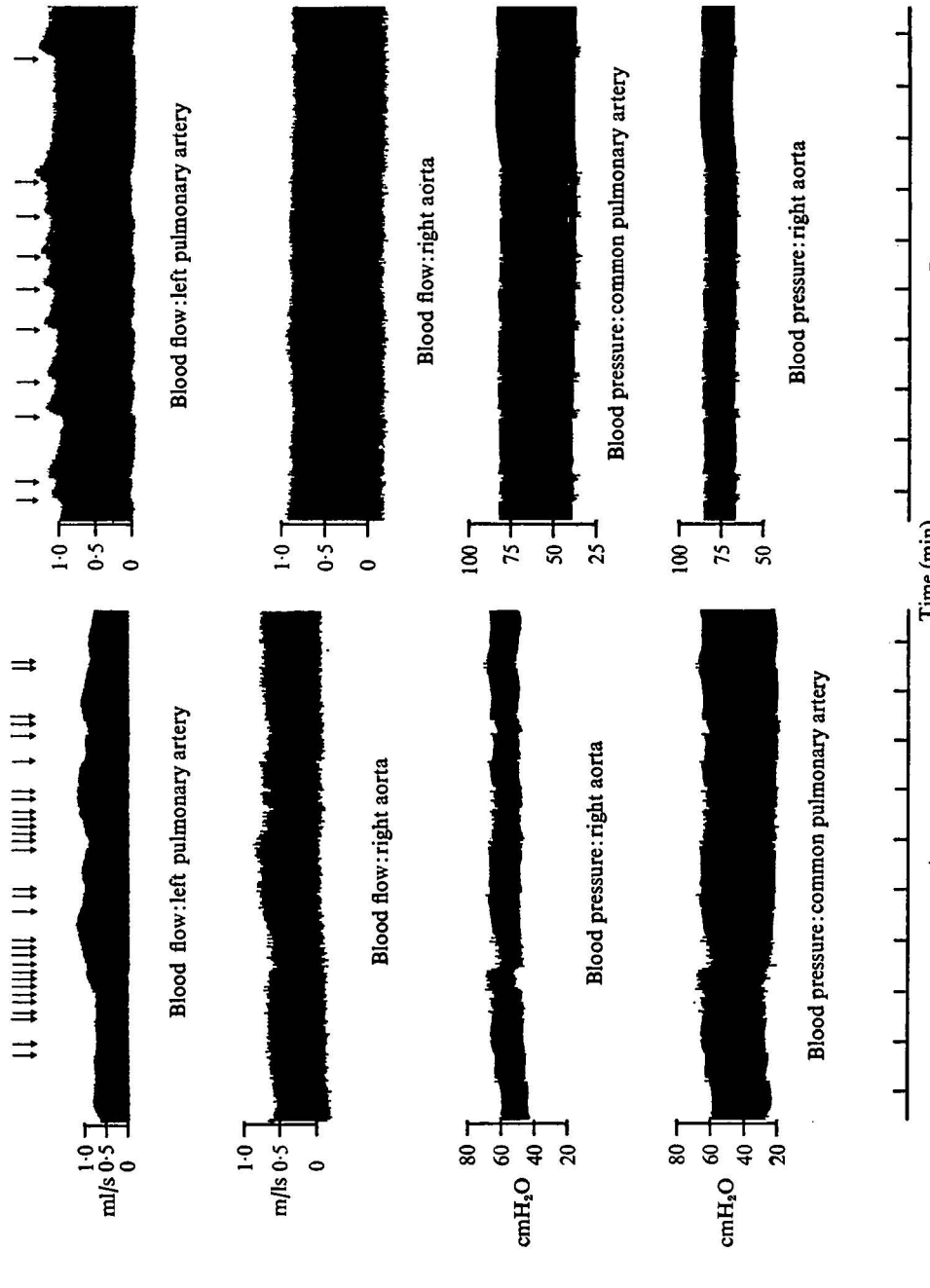

Fig. 9. Effect of lung ventilation on systemic and pulmonary pressures and flows in (A) unrestrained *Testudo* and (B) unrestrained *Pseudemys*. In (A) each arrow indicates a single breath. In (B) each arrow indicates the onset of a breathing series.

Table 2. *The effect of lung ventilation, apnoea and ventilation tachycardia on arterial blood flow in* Pseudemys scripta *and* Testudo graeca

(Measurements in the 'apnoea' column were made after a minimum of 5 min of voluntary apnoea in *Pseudemys* and after 45 s in *Testudo*. Measurements in the 'lung ventilation' column were made during or immediately after lung ventilation. Values presented are means ± 1 standard error. Systemic stroke flow was calculated as 2.75 × right aorta stroke flow based on extensive flow measurements from a specimen of *Testudo* (see Results). Thus, total cardiac output was calculated as: cardiac output = 2 (left pulmonary stroke flow) + 2·75 (right aorta stroke flow).)

	N	Apnoea duration		Heart rate (beats/min)	Right aorta		Left pulmonary artery		Estimated total cardiac output, *minute* flow (ml/min)	Increase in cardiac output with lung ventilation (%)
					Stroke flow (ml)	Minute flow (ml/min)	Stroke flow (ml)	Minute flow (ml/min)		
Pseudemys scripta exhibiting ventilation tachycardia	9	12 ± 9 (min)	Apnoea	11 ± 1·3	0·57 ± 0·05	6·72 ± 0·95	0·66 ± 0·11	7·38 ± 1·07	33·2	116%
			Lung ventilation	23 ± 1·0	0·40 ± 0·03	9·13 ± 0·72	0·99 ± 0·12	23·31 ± 3·00	71·7	
P. scripta not exhibiting ventilation tachycardia	19	15 ± 2 (min)	Apnoea	30 ± 1·1	0·38 ± 0·02	13·42 ± 0·84	0·55 ± 0·04	16·08 ± 1·59	69·1	27%
			Lung ventilation	30 ± 1·1	0·42 ± 0·01	14·80 ± 0·67	0·74 ± 0·07	23·61 ± 2·78	87·9	
Testudo graeca not exhibiting ventilation tachycardia	17	84 ± 12 (sec)	Apnoea	23 ± 0·5	0·50 ± 0·03	12·88 ± 1·01	0·83 ± 0·02	19·24 ± 0·63	73·9	18%
			Lung ventilation	24 ± 0·5	0·47 ± 0·02	11·97 ± 0·74	1·16 ± 0·08	27·15 ± 1·90	87·2	

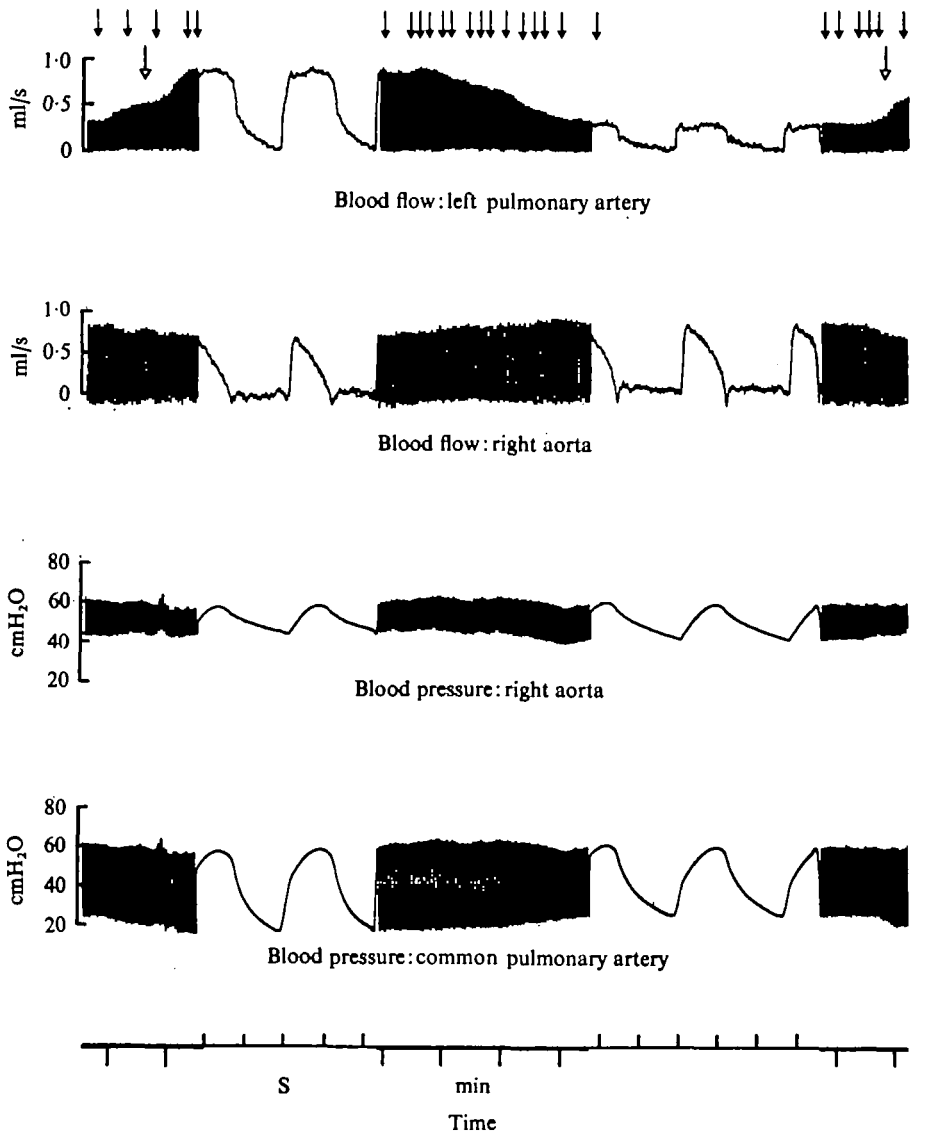

Fig. 10. Effect of locomotor activity on systemic pressures and flows in unrestrained *Testudo*. Each solid arrow indicates a single breath. At the open arrows a short burst of activity occurred.

apnoea. Student's paired-sample *t* test showed that stroke flows in the pulmonary artery during ventilation were significantly higher (at the 0·01 level) than those found during apnoea, in both the tachycardia and non-tachycardia animals (Table 2).

In most of the animals with a fairly uniform heart rate, systemic flow was usually unaffected by the variations in blood flow through the lungs (Fig. 9B). In a few preparations, reciprocal changes were seen in the two sides of the circulation with systemic flow decreasing during ventilation as pulmonary flow increased. In those animals showing a marked ventilation tachycardia the situation was more complicated since the increase in heart rate invariably put up minute flow in both

pulmonary and systemic vessels (Table 2). However, stroke flow in the pulmonary circulation was always affected more than that in the systemic and on some occasions the systemic stroke flow declined during lung ventilation. In both the tachycardia and non-tachycardia animals in Table 2, the values for stroke flow in the right aorta during lung ventilation were not significantly different from those determined during apnoea.

The profiles of the flow pulses from systemic and pulmonary vessels retained their characteristic differences during all the fluctuations in absolute flow levels (Figs. 5A, B, 10).

(ii) *Activity*

In both species activity of various types had substantial effects on cardiac output and blood flow. In *Pseudemys* in particular, movements of the body and head leading up to lung ventilation often caused both heart rate and stroke volume to increase before the breathing movements occurred (Fig. 8). Even such minor activity as clearing the nostrils of a water meniscus could affect blood flow, especially in the pulmonary circulation. The effects of the general level of locomotor activity could also be quite marked. In *Testudo*, for example, where the ventilation-induced changes in blood flow were quite small, movement around the experimental tank caused a considerable increase in pulmonary blood flow with a small reciprocal effect on systemic flow (Fig. 10).

DISCUSSION

During ventricular contraction, blood pressures are essentially the same throughout the ventricle and arterial arches of both *Pseudemys* and *Testudo*. Furthermore, pressure profiles within the three chambers of the ventricle are identical during the entire cardiac cycle. This could be achieved either by low resistance connexions permitting unimpeded flow between the chambers or, if the chambers are more or less separate and only high resistance connexions exist between them, by activity in the ventricular muscle being so closely adjusted to the impedance characteristics of the different outflow tracts that no intraventricular pressure gradients and flows will occur. The latter is a most unlikely possibility, particularly in view of the constant adjustments that are made in arterial flow, and the evidence suggests to us that the chelonian ventricle shows a functional as well as an anatomical continuity. The systemic and pulmonary circulations are therefore perfused by a single ventricular pump, a conclusion which applies to both ventilating and non-ventilating animals. We found no evidence for the separation of ventricular pumps during lung ventilation as described by White & Ross (1966).

Only two exceptions were found in which the pressures did not conform to the pattern discussed above. In one the controls were inadequate but, in the other, tests appeared to confirm the validity of the measurements shown in Fig. 3B. These results imply that under some circumstances a form of ventricular division can occur. As the animal on this occasion was heavily anaesthetized and all further attempts to repeat the result were unsuccessful, we conclude that such division is not normal.

In most of our experiments pulmonary arterial pressure between opening and closing of the pulmonary valves was very slightly but significantly depressed from

that in all other parts of the central arterial circulation. The results show that this is due to the resistance offered by the pulmonary outflow tract. The resistance is greatest during the later stages of systole when the pressure gradient is maximal but the rate of outflow is low. An increase in resistance towards the end of every cardiac cycle may be due to contraction of some part of the cardiac musculature restricting the dimensions of the outflow tract. Muscle of the reduced bulbus cordis which surrounds the base of the pulmonary artery seems to be appropriately placed to have such an effect. The small changes in overall gradient that can occur spontaneously or if the outflow region is mechanically stimulated suggest that some smooth muscle is also present in the outflow tract, perhaps in the initial segment of the pulmonary artery. If such muscle is present, however, it does not give rise to gradients larger than 1 or 2 cmH_2O in the normal animal. The values for the pressure drop across the pulmonary outflow tract suggest that it contributes about 5 % of the total resistance seen by the ventricle, the remaining 95 % being more peripherally situated.

Because the chelonian ventricle is apparently incapable of forming functionally separate pumps, the relative volume of blood entering the pulmonary or systemic circulation depends on the balance in the impedances offered by the two circuits. During ventilation the increases in pulmonary relative to systemic flow, in some cases in the absence of significant changes in central arterial blood pressure, are clearly caused by a selective pulmonary vasodilation. In other cases, especially in the animals showing ventilation tachycardia, quite marked changes in blood pressure occur and there are frequently variations in systemic blood flow. Even so, calculation shows that pulmonary vasodilation still constitutes the most significant peripheral change. For example, the general relationships can be seen if the effects of pulsatile flow, inertia and elasticity of the vessels are ignored and simple calculations of peripheral resistance (mean pressure/mean flow) are made for those *Pseudemys* showing ventilation tachycardia from which the data in Table 2 were obtained. During apnoea the mean resistance of the pulmonary circulation peripheral to the points of pressure and flow measurement in the left pulmonary artery is some $1{\cdot}52 \times 10^5$ dyn s cm^{-5}, but this falls to a value of $0{\cdot}60 \times 10^5$ dyn s cm^{-5} during and shortly after lung ventilation. The corresponding figures for the right aortic circulation are $2{\cdot}30 \times 10^5$ dyn s cm^{-5} during apnoea and $2{\cdot}01 \times 10^5$ dyn s cm^{-5} during ventilation. Both pulmonary and systemic vascular beds constrict during apnoea, especially that accompanying a dive, and dilate during lung ventilation, but the pulmonary changes are substantially the larger. The contribution made by the pulmonary outflow tract to the overall changes in resistance was unmeasurably small; no detectable increase in the systolic differences between systemic and pulmonary vessels was observed during apnoea. The major site of variation in pulmonary impedance is in the vascular bed of the lung and perhaps the smaller arteries (Berger, 1972) leading to it.

The independent variation of systemic and pulmonary impedances will obviously lead to quite considerable adjustments in flow patterns within the ventricle. Using the admittedly approximate factors given in Table 2 to calculate total pulmonary and systemic flows, some indication of the change in net shunt of blood entering the ventricle from right and left auricles can be obtained. During lung ventilation in *Pseudemys*, some 60–65 % of the cardiac output perfuses the pulmonary circulation. This can only be maintained if some of the blood returning from the lungs leaves the

ventricle and enters the pulmonary circulation again (left to right shunt). With the degree of imbalance given above, for example, at least 33–46 % of the blood in the left auricle would be returned directly to the lungs. These minimum figures would only be attained in the unlikely event of the entire output of the right auricle being conveyed to the pulmonary arteries. Similarly, during an apnoea of longer than 5 min in *Pseudemys*, only 45 % of the cardiac output flows into the pulmonary circulation and, in this case, at least 18 % of the blood in the right auricle must return directly to the systemic vessels (right-to-left shunt). These observations are in good agreement with estimations of intracardiac blood shunts in *Pseudemys* under similar experimental conditions made by White & Ross (1966). In *Testudo*, which shows much shorter periods of apnoea, a left-to-right shunt was continuously maintained since 53 % of the cardiac output perfused the pulmonary circulation during apnoea, increasing to 60–65 % during lung ventilation.

Our conclusions on the functional relationships within the heart suggest that, in the absence of separate ventricular pumps, mixing of blood from right to left auricles can occur during both ventricular filling and emptying. In these conditions it seems certain that shunts will occur in both directions and that the net figures indicated by flow measurements are the result of shunt in one direction being larger than that in the other. Even so the evidence from measurement of blood oxygen levels (Steggerda & Essex, 1957) and from dye dilution techniques (Millen *et al.* 1964) shows that shunts are not sufficiently large to eliminate completely any differences in blood from the right and left auricles as it is pumped by the ventricle to pulmonary and systemic arteries. These differences must be maintained by a laminar flow of blood in the ventricle, aided by internal structures such as the muscular ridges and the trabeculate nature of the ventricular wall. Ventricular function, as we propose it, is less complex than has been suggested by other authors, although, in conjunction with the moment to moment changes in the volume of blood flowing in to right and left auricles, it can still give rise to complicated and changing patterns of blood distribution in the ventricle. We would expect each of the four arteries (pulmonary artery, brachiocephalic artery, right aorta and left aorta) leaving the ventricle to bear a unique relationship to these patterns and therefore to contain different mixtures of blood from right and left auricles. The evaluation of total shunts in such a system is obviously complex. Experiments to test the hypothesis by examining the relationships between the arteries and the heart are now in progress.

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REFERENCES

BERGER, P. J. (1972). The vagal and sympathetic innervation of the isolated pulmonary artery of a lizard and a tortoise. *Comp. Gen. Pharmac.* **3**, 113–24.

BURGGREN, W. W. (1975). A quantitative analysis of ventilation tachycardia and its control in two chelonians *Pseudemys scripta* and *Testudo graeca*. *J. exp. Biol.* **63**, 367–80.

GANS, C. & HUGHES, G. M. (1967). The mechanism of lung ventilation in the Tortoise, *Testudo graeca* Linné. *J. exp. Biol.* **47**, 1–20.

HUNT, T. J. (1964). Anaesthesia of the tortoise. In *Small Animal Anaesthesia* (ed. O. Graham Jones). Oxford: Pergamon Press.

JOHANSEN, K., LENFANT, C. & HANSON, D. (1970). Phylogenetic development of pulmonary circulation. *Fedn Proc. Fedn Am. Socs exp. Biol.* **29**, 1135–40.

KAPLAN, H. M. (1969). Anaesthesia in amphibians and reptiles. *Fedn Proc. Fedn Am. Socs exp. Biol.* **28**, 1541–6.

KHALIL, F. & ZAKI, K. (1964). Distribution of blood in the ventricle and aortic arches in reptilia. *Z. vergl. Physiol.* **48**, 663–89.

MARCH, H. W. (1961). Persistence of a functioning bulbus cordis homologue in the turtle heart. *Am. J. Physiol.* **201**, 1109–12.

MATHUR, P. N. (1946). The anatomy of the reptilian heart. II. Serpentes, Testudinata and Loricata. *Proc. Indian Acad. Sci.* B **23**, 129–52.

MILLARD, R. W. & JOHANSEN, K. (1974). Ventricular outflow dynamics in the lizard, *Varanus niloticus*: Responses to hypoxia, hypercarbia and diving. *J. exp. Biol.* **60** (3), 871–80.

MILLEN, J. E., MURDAUGH, H. V., BAUER, C. B. & ROBIN, D. (1964). Circulatory adaptation to diving in the freshwater turtle. *Science, N.Y.* **145**, 591–3.

SHANNON, E. W. & WIGGERS, C. J. (1939). The dynamics of the frog and turtle hearts. The non-refractory phase of systole. *Am. J. Physiol.* **128**, 709–15.

SHELTON, G. (1975). Gas exchange, pulmonary blood supply, and the partially divided amphibian heart. *Proc. 50th Anniv. Meeting Soc. exp. Biol.* (in Press).

STEGGERDA, F. R. & ESSEX, H. E. (1957). Circulation and blood pressure in the great vessels and heart of the turtle *Chelydra serpentina*. *Am. J. Physiol.* **190**, 320–6.

TUCKER, V. A. (1966). Oxygen transport by the circulatory system of the green iguana (*Iguana iguana*) at different body temperatures. *J. exp. Biol.* **44**, 77–92.

WHITE, F. N. (1968). Functional anatomy of the heart of reptiles. *Am. Zoologist* **8**, 211–19.

WHITE, F. N. (1969). Redistribution of cardiac output in the diving alligator. *Copeia* 3, 567–70.

WHITE, F. N. (1970). Central vascular shunts and their control in reptiles. *Fedn Proc. Fedn Am. Socs exp. Biol.* **29**, 1149–53.

WHITE, F. N. & ROSS, G. (1965). Blood flow in turtles. *Nature, Lond.* **208**, 759–60.

WHITE, F. N. & ROSS, G. (1966). Circulatory changes during experimental diving in the turtle. *Am. J. Physiol.* **211**, 15–18.

WOODBURY, R. A. & ROBERTSON, G. G. (1942). The one ventricle pump and the pulmonary arterial pressure of the turtle: the influence of artificial acceleration of the heart, changes in temperature, haemorrhage and epinephrine. *Am. J. Physiol.* **137**, 628–36.