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GAS EXCHANGE AND TRANSPORT DURING INTERMITTENT BREATHING IN CHELONIAN REPTILES

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SUMMARY

1. The oxygen and carbon dioxide gas tensions in lung gas and blood from the central and peripheral arteries and veins have been measured in unrestrained, undisturbed turtles (*Pseudemys scripta*) and tortoises (*Testudo graeca*).

2. Lung and blood gas composition fluctuates widely with intermittent and irregular lung ventilation. The pulmonary gas exchange ratio, which progressively falls during apnoea to as low as 0·2–0·3 in *Pseudemys*, rises dramatically to over 1·5 during lung ventilation in both species. It is postulated that CO_2, which has been passively stored by entering the tissues along gas tension gradients during apnoea, becomes rapidly eliminated into the lungs during ventilation. In diving *Pseudemys* the lung has only a limited function as a CO_2 sink compared to the tissues, but the lung acts as a large oxygen store, which can be drawn upon during apnoea through periodic increases in lung perfusion.

3. Blood gas tensions in the various systemic arches reflect the proximity of the arch's origin to the systemic or pulmonary venous blood streams in the ventricle. Thus, the brachiocephalic artery and right aorta have identical blood gas compositions while the composition of the left aorta is intermediate between these and that in the pulmonary. These relationships are unaffected by normal intermittent breathing. However, this does affect both the origin and composition of systemic and pulmonary arterial blood, such that the greatest proportion of oxygenated blood perfuses the systemic vascular bed during lung ventilation, while the greatest proportion of deoxygenated blood perfuses the lungs during apnoea.

4. These data are discussed in the light of the marked cardiovascular adjustments to intermittent breathing, which occur in chelonian reptiles.

INTRODUCTION

In most fish, birds and mammals, ventilation of the gills or lungs is a continuous process. Perfusion of the gas exchanger is closely matched with its ventilation, and gas transport to and from the tissues occurs within a narrow and well defined range of internal conditions. The control mechanisms involved in the maintenance of this homeostasis are well understood in birds and mammals. Many amphibians and reptiles,

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however, ventilate their lungs intermittently and undergo fluctuations in respiratory gas tensions, acid-base balance, and metabolic end-product concentrations which birds and mammals could not tolerate. The mechanisms regulating the limits of these fluctuations are largely unknown.

Many aquatic chelonians are capable of extensive periods of apnoea between bursts of breaths and even terrestrial species do not breathe continuously (Shaw & Baldwin, 1935; McCutcheon, 1943; Randall, Stullken & Hiestand, 1944; Boyer, 1966; Gans & Hughes, 1967; Lenfant *et al.* 1970; Burggren, Glass & Johansen, 1977). Although some aquatic chelonians have a limited potential for gas exchange with the water surrounding them (Girgis, 1961; Belkin, 1968*a*; Jackson, Allen & Strupp, 1976), in most aquatic and all terrestrial species breath-holding effectively stops gas exchange with the environment. Predictably, these reptiles show large internal variations in respiratory gas levels during normal intermittent breathing (Lenfant *et al.* 1970) or forced diving (Jackson & Silverblatt, 1974; Penney, 1974).

Interpretation of the functional relationships between gas exchanger, blood and tissues during the progressive apnoea following lung ventilation and of the processes related to the initiation of the next breath is complicated in chelonians by two major factors. First of these is the outstanding ability of chelonian tissues to metabolize at low levels during progressive oxygen depletion (Belkin, 1968*b*) and to survive long periods of total anoxia, presumably by means of anaerobic glycolysis (Robin *et al.* 1964; Belkin, 1968*a*). The second factor is a capacity for adjustment within the circulatory system, found generally among a wide variety of animals, such that during apnoea arterial blood from a substantially reduced cardiac output is preferentially directed within the systemic circulation (see White, 1976; Shelton & Burggren, 1976). The extent to which cells of different tissues contribute to or are exposed to the physiological consequences of apnoea is therefore subject to wide variation in the Chelonia. It thus is not surprising that the time courses and rates of change of respiratory phenomena such as tissue metabolism, blood gas transport or pulmonary gas exchange are difficult to establish.

Recent measurements of blood flow in the major arteries leaving the heart have shown that, in unrestrained chelonians, the lung is one of the regions whose perfusion is most profoundly influenced by the change from active lung ventilation to apnoea (White & Ross, 1966; Shelton & Burggren, 1976; Burggren *et al.* 1977). Such a change results in a dramatic fall in blood flow to the unventilated lung because of vascoconstriction in the pulmonary vasculature at a number of different sites (Burggren, 1977). These observations have considerable bearing on the long-standing problem of blood flow in the incompletely divided reptilian heart. Radiographic techniques (Prakash, 1952; Foxon, Griffith & Price, 1953; Johansen & Hol, 1960) and the measurement of oxygen and carbon dioxide contents of venous and arterial blood (Steggerda & Essex, 1957; White, 1959; Khalil & Zaki, 1964; Tucker, 1966) have produced convincing evidence of substantial separation of oxygenated and deoxygenated blood within the anatomically complex ventricle. However, it seems clear that a static view of the central circulation, derived from pithed or anaesthetized animals often with artificial ventilation, is inadequate when specific values are allocated to the degree of blood separation and left-to-right or right-to-left shunt. Continuous adjustment of blood flow in the

pulmonary circuit, as the lungs are ventilated or apnoea progresses, will obviously change the separation of blood between left and right sides of the heart.

In the present investigation measurements are made of the respiratory gas tensions in the lungs, central arteries and veins of unanaesthetized, unrestrained, turtles and tortoises to determine the changes occurring as the animals breathe in a normal, intermittent fashion. The results are used to examine the problem of blood flow through the heart and the concept of variable shunts.

METHODS

Experiments were carried out at 18°–20 °C on a total of 27 aquatic turtles (*Pseudemys scripta*) and 18 terrestrial tortoises (*Testudo graeca*). All animals weighed between 700 and 1100 g. Measurements of gas tensions were made on samples of blood or lung gas withdrawn from the animals through chronically implanted cannulae. The surgery to implant the cannulae was carried out after torpor had been induced by exposing the animals to low temperature (1 °C) for 12–15 h. After warming to room temperature, *Pseudemys* was placed in a small tank of water in which voluntary diving was possible, while *Testudo* was housed in a small, dry cage. Each animal was allowed to recover for at least 24 h under these conditions before measurements were begun and in all the experiments the unrestrained animals breathed voluntarily.

Lung cannulation

The lung cannula was implanted in an anterior lateral chamber (gas tensions are effectively identical in all the large lateral chambers (Burggren, Glass & Johansen, 1978)) of either the right or left lung. Details of lung cannulation have been given elsewhere (Burggren, 1975). Lung cannulae remained patent for several days and subsequent dissection revealed little or no contusion or oedema.

Cannulation of arteries and veins

In some animals the femoral artery alone was occlusively cannulated (polythene PP60) in an upstream direction through an incision made in the left or right thigh. The incision was closed using interrupted sutures and 200 i.u./kg body weight of heparin was injected immediately. A similar dose of heparin was administered daily.

In other animals, central arteries and veins were cannulated. Full details of surgical procedures have been given elsewhere (Shelton & Burggren, 1976). The heart and central blood vessels were exposed by removing a 4 cm square piece of the plastron. Various combinations of the left and right aortae, the brachiocephalic and pulmonary arteries, and the vena cava and pulmonary vein were non-occlusively cannulated in an upstream direction with PP25 to PP60 polythene tubing. The cannulae were anchored in position with a loop of surgical silk tied into the adventitia of the blood vessel. They were guided out through a small groove cut in the excised piece of plastron which was then sealed back into position with epoxy resin. The lung was also cannulated in these experimental animals. Blood loss during these procedures was usually negligible. Heparin was injected as for the femoral cannulations.

Determination of gas tensions

Lung gas was withdrawn via the implanted cannula into an air-tight glass syringe and the partial pressures of oxygen (P_{O_2}) and carbon dioxide (P_{CO_2}) measured with a Radiometer BMS3 gas analyser. Blood samples were taken by connecting a blood cannula to the inlet of a pair of serially connected thermostatted cells containing oxygen and carbon dioxide electrodes (all Radiometer). The electrode outputs were connected to a Radiometer PHM71 or a Beckman 160 physiological gas analyzer. The electrodes were calibrated frequently during an experiment using humidified gas mixtures. A glass syringe filled with CO_2 calibration gas close to *in vivo* P_{CO_2} levels was attached to the outlet of the pair of thermostatted cells. Arterial blood was sampled by allowing it to flow under arterial pressure through the system, displacing calibration gas into the syringe. Sufficiently large samples were drawn to ensure that the electrode faces encountered fresh arterial blood. After the measurements had been made, a slight positive pressure was applied to the syringe to return the blood sample to the circulation, provided that no gas bubbles were present in the sample. Venous blood was sampled by gently applying a small negative pressure with the syringe to draw blood into the system. Repeated samples could be taken in this way from arterial and venous systems without undue blood loss or dilution. Great care was taken to ensure that small gas bubbles were never introduced into the animal, which could have resulted in emboli.

RESULTS

1. *Pulmonary and systemic arterial gas tensions*

In *Pseudemys* normal periods of apnoea last for several minutes and are punctuated by breathing series consisting of 5–10 ventilations, whereas in *Testudo* the ventilations are single and separated by periods of apnoea usually less than a minute in duration (Burggren, 1975). The effects of these patterns on respiratory gases in lungs and blood are substantially different in the two species.

Pseudemys scripta

Representative changes in respiratory gas tensions found in the lungs and femoral artery during voluntary, intermittent diving in a single turtle are illustrated in Fig. 1. Clearly, there were large variations in duration of apnoea and the levels to which gas tensions changed before the next breath was taken. In an effort to describe the general changes which occur for a given species, measurements of lung gas and femoral arterial blood were made repeatedly during the course of 35 voluntary dives in 8 *Pseudemys* and these data then pooled. The mean values of respiratory gas tensions recorded as apnoea progressed, usually during a dive, are plotted in Fig. 2A. Although differences from this pattern were occasionally seen (Fig. 4, for example), the mean levels to which the gas tensions changed were clearly a function of time. The rates of change in both lung and femoral artery were greatest during the early stages of a dive and fell quite substantially if the dive went on for more than 15 min. The measured values of gas tensions for oxygen and carbon dioxide in alveolar gas showed progressively greater reductions in P_{O_2} than tee corresponding increases in P_{CO_2}, so that carbon dioxide elimi-

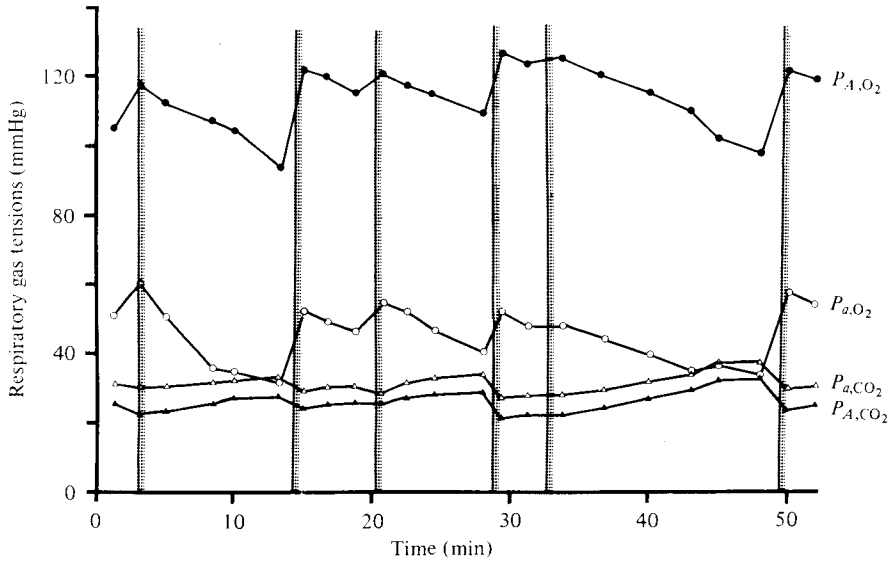

Fig. 1. Fluctuations in lung O_2 and CO_2 gas tensions (P_{A,O_2} and P_{A,CO_2}) and femoral artery O_2 and CO_2 gas tensions (P_{a,O_2} and P_{a,CO_2}) during 1 h of intermittent breathing in a freely diving, unrestrained 0·97 kg *Pseudemys scripta*. Brief bouts of surfacing and lung ventilation are indicated by the shaded vertical bars.

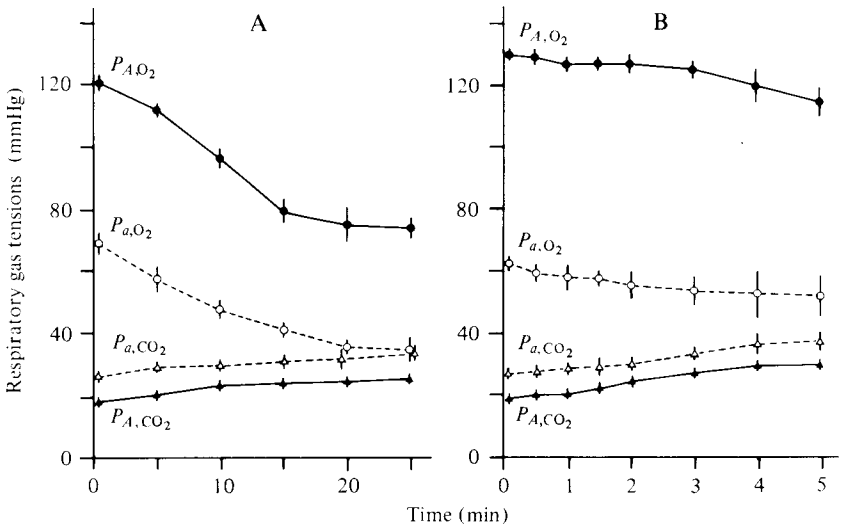

Fig. 2. Changes in lung gas and femoral artery blood P_{O_2} and P_{CO_2} during progressive apnoea in (A) *Pseudemys scripta* and (B) *Testudo graeca*. Values given, which are means ± 1 S.E., were determined in eight freely diving turtles and six unrestrained tortoises.

nation into the lung was less than oxygen uptake from it (Fig. 3). As a consequence lung volume must have decreased during a dive. The oxygen-carbon dioxide diagram (Fig. 3) shows that the pulmonary exchange ratio, $\dot{V}_{CO_2}/\dot{V}_{O_2}$, fell to values of about 0·3 during the longer dives and went up to 1·5 or 2·0 during the brief periods of lung ventilation.

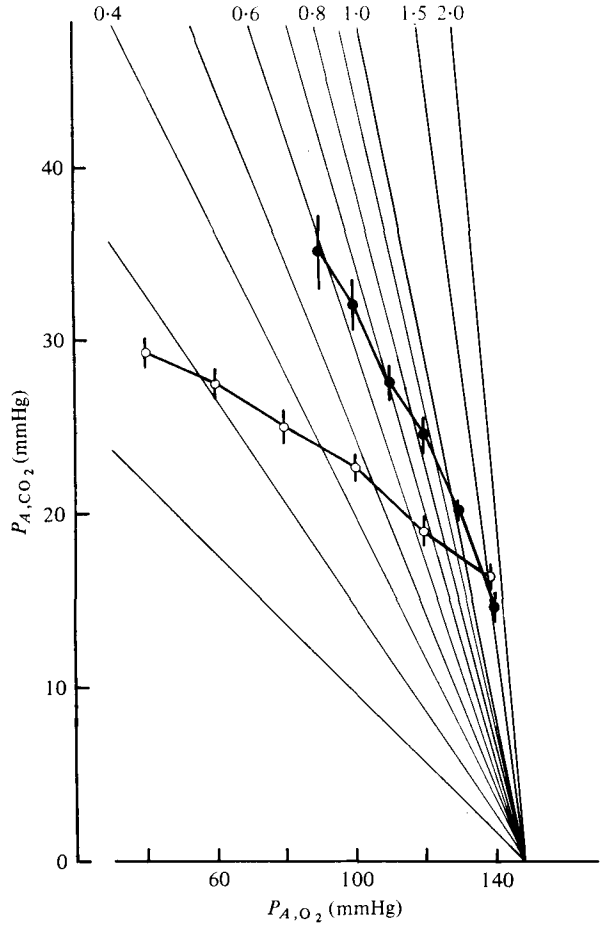

Fig. 3. Pulmonary gas exchange quotient (R_{PE}) determined during voluntary intermittent lung ventilation in eight *Pseudemys scripta* (open circles) and five *Testudo graeca* (solid circles). Mean values ± 1 S.E. are presented. Also indicated are the lines corresponding to values of R_{PE} from 0·2 to 2·0.

A substantial difference in P_{O_2} of 45–50 mmHg was found between lung gas and femoral artery blood during breathing, although this decreased somewhat during apnoea (Fig. 2A). The P_{CO_2} difference from blood to lung gas was much smaller (6–12 mmHg) and showed no measurable change during apnoea. After a dive, O_2 tensions in blood and lungs were usually restored to the high levels shown in Fig. 2A in an initial breathing period of 10–15 breaths, and the next dive then followed immediately. On some occasions a short period of apnoea at the surface was followed by a second set of breathing movements which was necessary to complete the recovery to pre-dive levels.

Though there was a lot of variation in measured tensions from animal to animal and in an individual from dive to dive, the levels reached were usually a fairly simple function of the dive duration, as Figs. 1 and 2A suggest. However, in about 20% of all the dives studied and commonly in those of more than 30 min duration, a different pattern

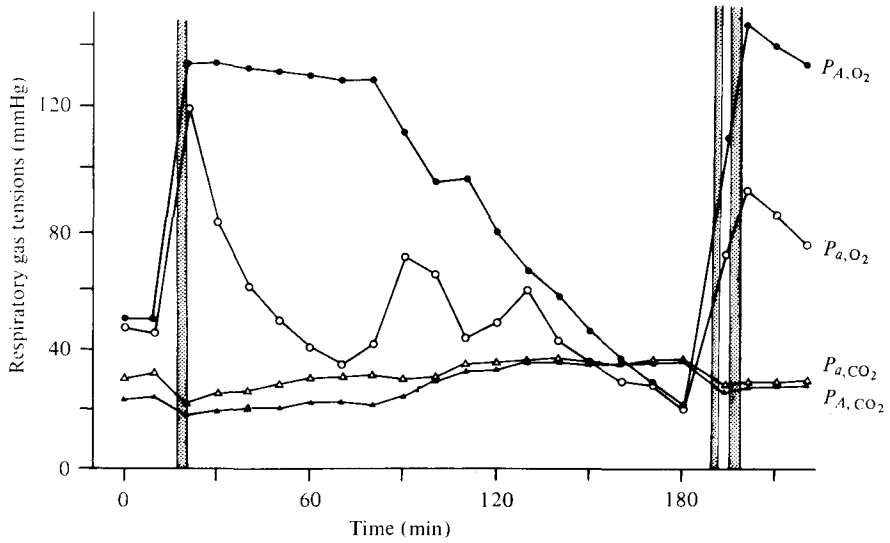

Fig. 4. Changes in lung gas and femoral artery blood P_{O_2} and P_{CO_2} during an extended voluntary dive in a 1·20 kg *Pseudemys scripta*. Shaded vertical bars represent periods of active lung ventilation.

emerged in which the respiratory gas tensions did not show uniform change (Fig. 4). At the onset of the dive arterial oxygen tension (P_{a,O_2}) fell rapidly while alveolar values (P_{A,O_2}) changed very little. After 20–40 min into the dive the fall in P_{A,O_2} was greatly accelerated and the P_{a,O_2} actually increased in value. After a short time the relationships evident at the start of the dive were restored. Similar changes were seen in the P_{CO_2} levels. This type of oscillation in the magnitude of P_{O_2} and P_{CO_2} differences between lung and arterial blood was often repeated several times in the course of a very long dive. From the outset dives seemed to be either of the type illustrated in Fig. 1 or that seen in Fig. 4; that is, transition from one pattern to the other was never seen during a single dive.

Testudo gracea

Since *Testudo* breathes more regularly and frequently than *Pseudemys*, considerably smaller fluctuations were found in the respiratory gas tensions measured in both blood and lungs. Changes in the mean values, as recorded during 80 periods of apnoea in six tortoises, are plotted in Fig. 2B. Gas tensions in lung and arterial blood at the beginning of a period of apnoea were approximately the same for *Testudo* as for *Pseudemys*, and oxygen tensions fell at similar rates in both animals during apnoea (2–3 mmHg/minute). The rate of increase in P_{CO_2} of both lung gas and arterial blood was substantially greater in *Testudo*, however, and the pulmonary exchange ratio therefore varied over a narrower range at higher values in this species (Fig. 3). The 60–70 mmHg P_{O_2} differences between lung and blood and the 8–12 mmHg P_{CO_2} difference varied little during breathing and apnoea. When periods of apnoea were shorter than 1 min, as they usually were in *Testudo*, a single breath would restore lung and arterial gas tensions to normal air-breathing values. After longer periods of apnoea, two or more breaths,

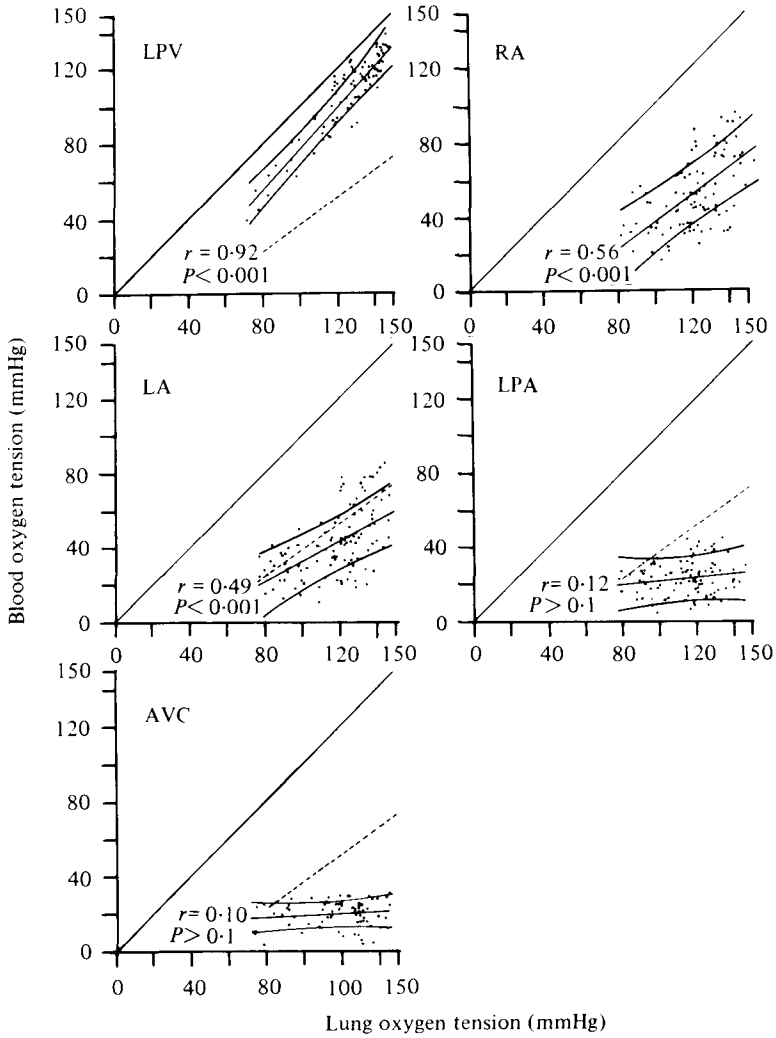

Fig. 5. Changes in arterial and venous blood oxygen gas tensions accompanying changes in lung gas P_{O_2} during intermittent breathing in nine freely diving *Pseudemys scripta*. Lung P_{O_2} is plotted on the abscissa. The iso-oxygen lines run at 45° from the origin. The regression line and 95% confidence interval is presented for each set of data, as is the coefficient of correlation, *r*, and the significance level of the slope of the regression line. The dashed line on each graph represents the linear regression line calculated for the P_{O_2} values of blood from the right aorta. LPV, left pulmonary vein; RA, right aorta; LA, left aorta; LPA, left pulmonary artery; AVC, anterior vena cava.

usually taken at a frequency not obviously greater than normal, were needed to restore the tensions to the usual breathing levels.

2. *Central arterial and venous gas tensions*

The samples from which data in this section are derived were taken during periods of apnoea and lung ventilation in six unrestrained *Testudo* and nine freely diving *Pseudemys*. In Figs. 5–8 gas tensions in blood from central arteries and veins are plotted against tensions in gas samples taken simultaneously from the lung.

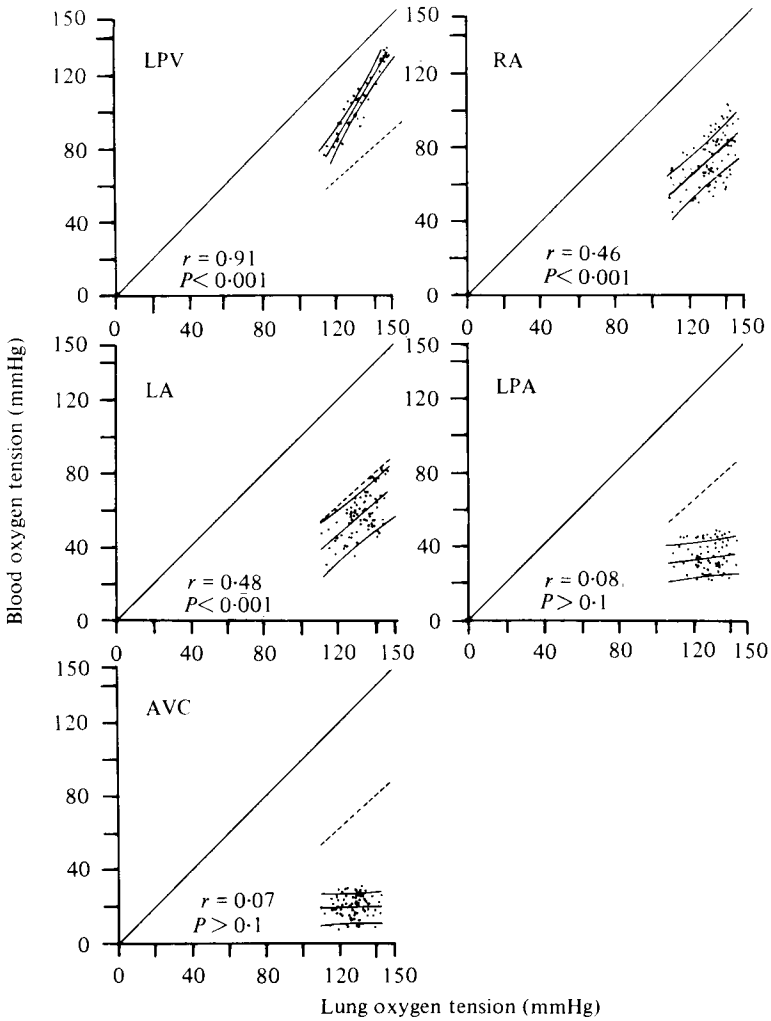

Fig. 6. Changes in arterial and venous blood oxygen gas tensions accompanying changes in P_{A,O_2} during intermittent breathing in six unrestrained *Testudo graeca*. See Fig. 5 for explanation of graph labels.

Oxygen tension

Though the range of fluctuations in oxygen tension is smaller in *Testudo* than it is in *Pseudemys*, the general relationships between arterial and venous samples is very similar in both species. The largest fluctuations in P_{O_2} were evident in the pulmonary veins, with the rate of change in pulmonary venous P_{O_2} per unit change in lung P_{O_2} significantly exceeding that in the left and right aorta (i.e. the slope of the regression line for pulmonary venous blood was greater than that of the other blood vessels at a significance level of at least $P < 0{\cdot}05$ for *Pseudemys* and $P < 0{\cdot}01$ for *Testudo* (Figs. 5 and 6)). In *Testudo*, though not in *Pseudemys*, there was also a significant increase in the P_{O_2} gradient from lung gas to blood as apnoea progressed, at least in that part of the pulmonary capillary closest to the vein (slope of the regression line for pulmonary venous blood in *Testudo* was significantly greater than 1 at the $P < 0{\cdot}01$ level). However, in

both species at the arterial end of the capillary the gradient progressively decreased, since apnoea produced no significant change in pulmonary artery P_{O_2}. Hence, the mean gradient from lung to blood almost certainly declined as apnoea progressed, as did the quantity of oxygen transferred (see below). Because of extensive blood shunts within the heart, P_{O_2}s in the right aorta were 20–50 mmHg lower than those in the pulmonary vein, and fluctuated over a smaller range.

Analysis of 62 paired blood samples drawn simultaneously from the right aorta and the brachiocephalic artery (leading from the heart to subclavian and carotid circulations) showed that there was no significant difference ($P > 0{\cdot}1$) in P_{O_2} of the blood from these two major arterial trunks in either *Testudo* or *Pseudemys*.

In 97 paired samples drawn simultaneously from right and left aortae, however, blood from the former had a P_{O_2} which was consistently 5–15 mmHg higher than that in blood from the latter ($P < 0{\cdot}01$) over a range of arterial tension from 20 to 110 mmHg. This indirect evidence for a difference in oxygen content of blood from the two aortae is especially significant during apnoea when the arterial tensions begin to move onto the steeper regions of the dissociation curve and small P_{O_2} differences are accompanied by large differences in O_2 content. Even during lung ventilation differences in oxygen content must still exist. Using blood data determined *in vitro* for *Pseudemys* and *Testudo* (Burggren, Hahn & Foëx, 1977), average values for the oxygen content of blood in the right aorta would be 7·7 vol. % for the former and 8·9 vol. % for the latter, during active lung ventilation with a lung P_{O_2} of 130 mmHg. Similar figures for the left aorta would be 6·9 and 8·3 vol. %. Therefore under all conditions in unanaesthetized animals the left aorta receives significantly greater proportions of venous blood (from the right auricle) than the right aorta. The results of Steggerda & Essex (1957), who frequently found identical oxygen contents in the two aortae of *Chelydra*, could be attributed to the fact that they worked on pithed, artificially ventilated animals. If any of the mechanisms resulting in separation of oxygenated and deoxygenated blood as it flows through the heart were to be actively controlled, destruction of the central nervous system could be expected to have profound effects on arterial blood gas tensions.

The lowest oxygen tensions were found in systemic venous blood sampled from the anterior vena cava. Venous P_{O_2} was effectively independent of ventilatory events, even in single individuals, and the pooled data in Figs. 5 and 6 gave regression lines whose slopes were not significantly different from zero. Even if the very small effect of increased carbon dioxide in lowering oxygen content is taken into account, it is clear that tissue extraction during apnoea does not lead to substantially lower oxygen levels in venous blood. The capacity of venous blood to act as an oxygen store therefore is not exploited during apnoea in these animals. Since apnoea leads to a major reduction in the oxygen content of arterial blood, delivery of oxygen to the tissues by a unit volume of blood must gradually decrease. In addition, cardiac output falls considerably (Shelton & Burggren, 1976), reducing oxygen delivery even further. The P_{O_2} of blood in the pulmonary artery also was not changed significantly during apnoea, but remained 5–10 mmHg higher than that in the anterior vena cava. Venous blood from the vena cava, in its passage through the heart to the pulmonary artery, must therefore be mixed with some oxygenated blood from the left auricle.

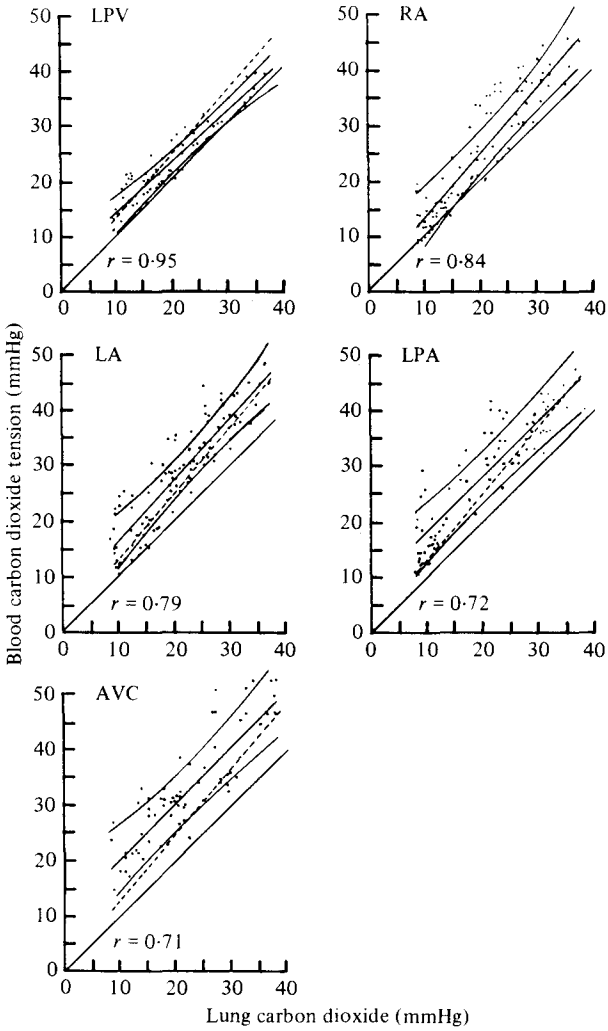

Fig. 7. Changes in arterial and venous blood carbon dioxide gas tensions accompanying changes in P_{A,CO_2} during intermittent breathing in nine freely diving *Pseudemys scripta*. See Fig. 5 for explanation of graph labels. $P < 0{\cdot}001$ for the significance level of the slope of all regression lines.

Carbon dioxide tension

The P_{CO_2} values plotted in Figs. 7 and 8 were determined in the same samples taken from lungs and blood as were used for the oxygen measurements. During apnoea the range over which the P_{CO_2} increased in both blood and alveolar gas was much smaller than the simultaneous fall in P_{O_2}, hence the decreasing pulmonary exchange ratio (Fig. 3). Because of the high solubility of carbon dioxide in tissue fluids and in blood, the diffusion gradients from blood to alveolar air and, presumably, from tissues to blood were also small, and the P_{CO_2} differences in blood samples from various parts of the circulation were not very marked. Nevertheless the general relationships between carbon dioxide tensions in the arterial and venous systems of both *Pseudemys* and

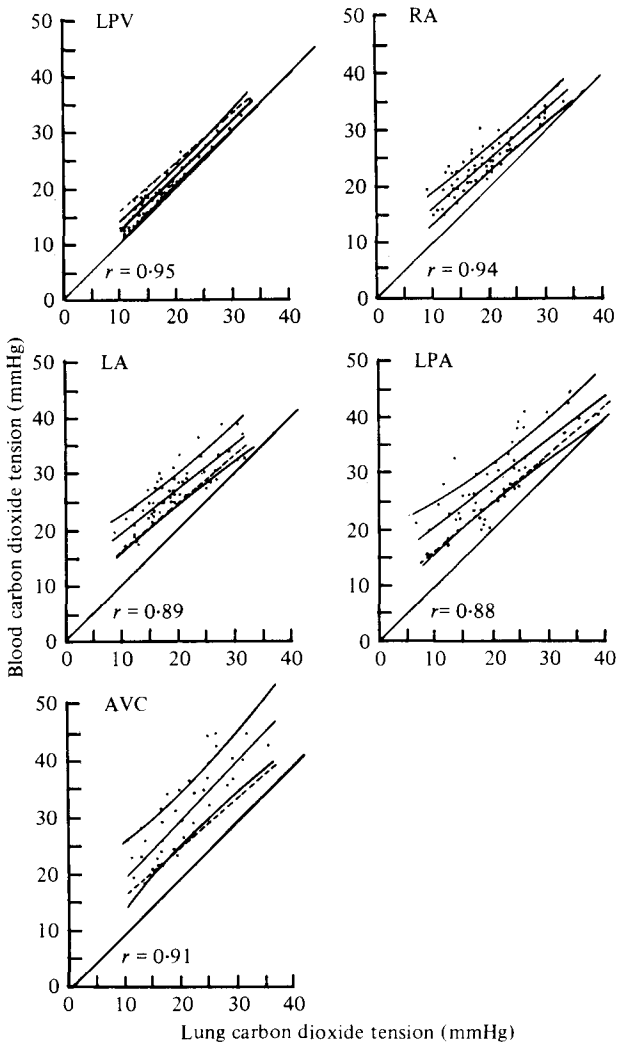

Fig. 8. Changes in arterial and venous blood carbon dioxide tensions accompanying changes in P_{A,CO_2} during intermittent breathing in six unrestrained *Testudo graeca*. See Fig. 5 for explanation of graph labels. $P < 0{\cdot}001$ for the significance level of the slope of all regression lines.

Testudo are consistent with the oxygen determinations described above. P_{CO_2} was greatest in blood from the anterior vena cava and left-to-right shunting in the heart produced slightly lower levels in the pulmonary artery. Blood in the pulmonary vein had the lowest P_{CO_2} and right-to-left shunting caused blood in right aorta and brachiocephalic arteries to show somewhat higher but identical tensions while blood from the left aorta showed slightly higher tensions still. As apnoea progressed in both the turtle and tortoise, P_{CO_2} increased at equivalent rates in all of the blood vessels and in the lungs (slopes of the regression lines in Fig. 7 and 8 were not significantly different from each other and from 1). Thus, the tissues, the blood and the lungs are all being used as regions for storage of carbon dioxide during apnoea.

DISCUSSION

Pulmonary gas exchange

Fluctuations in the respiratory gas tensions in the lungs of *Pseudemys* and *Testudo*, though of a variable nature, are very closely correlated with ventilatory events (Fig. 1). The marked changes in R_{PE} which develop during intermittent breathing in the turtles and tortoises show that relationships between O_2 and CO_2 exchange in the lungs are not simple. Proportionately more CO_2 than O_2 is exchanged by the lungs when the animal is breathing, so that values of R_{PE} are greater than 1, whereas during apnoea the R_{PE} falls progressively to values considerably less than 1 (Fig. 3).

There are two possible mechanisms which can explain these large variations in pulmonary gas exchange. The first is a progressive development of cutaneous CO_2 elimination, which as Lenfant *et al.* (1970) have suggested, could account for a decrease in R_{PE} during breath-holding. This hypothesis finds some support in the observation that the pulmonary gas exchange quotient remains considerably higher in *Testudo graeca* than in *Pseudemys scripta* (Fig. 3) or *Chelys fimbriata* (Lenfant *et al.* 1970) as apnoea progresses. There probably is much less potential for any cutaneous elimination of CO_2 in the terrestrial tortoise, which has a thick, dry and leathery integument compared with aquatic Chelonia.

It is difficult, however, to envisage how a mechanism involving cutaneous exchange could reasonably account for values of R_{PE} greater than 1 during lung ventilation.

A second, more likely mechanism which we postulate involves a considerable redistribution of O_2 and CO_2 between different body compartments such as the lungs, arterial and venous blood, and the tissues as gas tensions change. Estimates of O_2 and CO_2 movements during apnoea for a 1 kg *Pseudemys* experiencing an alveolar P_{O_2} drop from an air breathing value of 120 mmHg to a value during apnoea of 80 mmHg are in Table 1. These calculations are based on our measurements for gas tension changes in the lungs, arteries and veins and on realistic assumptions for blood tissue and lung volumes (see legend, Table 1). CO_2 solubilities in tissue fluid and blood are very much higher than those of oxygen. Thus, whereas less than 2% of the O_2 required during apnoea will come from the dissolved O_2 stores in the tissues themselves, fully 48% of the metabolically produced CO_2 will remain stored in various forms in the tissues until the large gas tension gradients favouring its rapid removal to the blood and lungs arise with the return of ventilation. Similarly, additional CO_2 becomes stored in the arterial and venous blood in the form of dissolved molecular CO_2, carbamino compounds, and bicarbonate ions as some CO_2 from the tissues moves along a gas tension gradient into the blood during apnoea, Because of the large solubility for CO_2 of the intervening blood and tissues, only 10–15% of the CO_2 produced during a normal period of apnoea will eventually be transported by pulmonary arterial blood to the lung gas store.

During apnoea the blood is functioning mainly to transport pulmonary oxygen rather than store this gas, however, with only 10% of the total O_2 consumed during apnoea coming directly from the O_2 initially contained in the erythrocytes themselves. At the same time, oxygen tension gradients favour a continuous transfer into the blood of pulmonary oxygen, which constitutes almost 90% of the O_2 metabolized during

Table 1. *Calculated oxygen and carbon dioxide transfer in the lungs, blood and tissue of a 1 kg* Pseudemys scripta *at 20 °C during a period of apnoea, which lowers the* P_{A,O_2} *from 120 to 80 mmHg and raises the* P_{A,CO_2} *from 28 to 35 mmHg.*

These calculations were based upon the assumption that a 1 kg turtle had a 100 ml lung volume, 500 ml of tissue which could constitute a usable short-term gas pool, and a 90 ml blood volume, of which 75% was 'venous' or partially deoxygenated blood. (No allowances were made for bone marrow or myoglobin). It was further assumed that the blood oxygen capacity was 8·7 vol % and the blood oxygen and carbon dioxide curves were as those described for this species elsewhere (Wilson, 1939, Burggren *et al.* 1977). Respiratory gas tensions in the blood and other tissues at the beginning and end of apnoea were estimated from the relationships between lung gases and blood given in Figs. 5 and 7.

	O_2 content (ml)				O_2 removed	CO_2 content (ml)				CO_2 added	Gas transfer
Site	P_{A,O_2} 120		P_{A,O_2} 80		(ml)	P_{A,CO_2} 28		P_{A,CO_2} 35		(ml)	quotient
Lungs	15·8	−	10·5	=	5·3	3·8	−	4·7	=	0·9	0·2
'Arterial' blood	1·7	−	1·4	=	0·3	18·5	−	19·1	=	0·6	2·0
'Venous' blood	2·7	−	2·4	=	0·3	55·3	−	57·2	=	1·9	6·3
Other tissues	0·4	−	0·3	=	0·1	12·0	−	15·1	=	3·1	31·0
Total	20·6	−	14·6	=	6·0	89·6	−	96·1	=	6·5	1·1

non-ventilatory periods. The consequence of the different O_2 and CO_2 solubilities of the various body compartments is that, with an overall gas tension gradient from lungs to tissues for O_2 and from tissues to lungs for CO_2, highly disproportionate quantities of O_2 and CO_2 will be removed from and added to these compartments during apnoea, as is clearly evident in Table 1.

With the onset of lung ventilation, the pulmonary and the comparatively meagre tissue and blood oxygen stores are replenished. The CO_2 tension gradient from the tissues to the lungs is also considerably increased, particularly after larger periods of apnoea, and as a result of the great solubility coefficient of the tissue and blood for CO_2 there will be a large and rapid divestment of CO_2 in these body compartments to the lungs. Since only very little CO_2 was excreted into the lungs during apnoea, about 85% of the total amount of CO_2 produced must then be rapidly transferred to the lungs and excreted during the brief period of ventilation, which occupies only 15% of the total activity of the turtle (Burggren, 1975).

Although the data in Table 1 are clearly approximations based on many assumptions, they do illustrate the major reason for a drastic fall in pulmonary R_{PE} during breath-holding and its elevation above 1 when breathing begins again.

During a dive the lungs of *Pseudemys* clearly function as an O_2 store. Since pulmonary perfusion can be regulated and usually decreases substantially during periods of apnoea in chelonian reptiles (see White, 1976; Shelton & Burggren, 1976; Burggren *et al.* 1977), the animal may exert considerable control over the rate of depletion of this store. This is corroborated by those experiments in which the rate of fall of lung oxygen varied enormously (Fig. 4). Most animals showed a more continuous depletion of lung oxygen, however, more closely represented by the mean values in Fig. 2. The reason for these two different patterns of lung gas and arterial blood gas tension changes during

diving in *Pseudemys* is at present unknown. Nonetheless, based partly on *in vitro* data for blood affinities (Burggren *et al.* 1977), in either situation the P_{O_2} of systemic arterial blood remains sufficiently high to afford nearly full Hb-O_2 saturation during all but the longest periods of apnoea.

Pulmonary exchange of oxygen thus can continue more or less at any time during lung ventilation or apnoea, whereas pulmonary CO_2 exchange is much more cyclical, occurring only when large gas tension gradients during the short breathing intervals favour the rapid movement of CO_2 from the blood and tissue stores.

Substantial differences in respiratory gas tensions were measured between lung gas and arterial blood during intermittent ventilation in *Pseudemys* and *Testudo*, as has been reported in other investigations of chelonians (Wilson, 1939; Lenfant *et al.* 1970). These differences result both from arterial-venous shunting within the functionally undivided chelonian ventricle and from a failure of pulmonary venous blood to reach equilibrium with lung gas. The latter could arise for a number of reasons. For example, significant barriers to gas diffusion in the lung could account in part for the gas tension differences between lung gas and pulmonary venous blood. Other factors involve inappropriate alveolar ventilation/perfusion ratios. Alveolar dead space, from the viewpoint of alveolar blood flow, represents hypoperfusion of some alveoli, while physiological shunting ('venous admixture') represents a hyperperfusion of other alveoli. Either of these conditions also will produce a difference in pulmonary venous P_{O_2} from the 'ideal value' that would be achieved if ventilation and perfusion of all lung units was perfectly matched (West, 1977). Pulmonary venous blood admixture can be calculated from measurements of the gas tensions of inspired and expired gas and pulmonary venous blood, using the 'ideal' gas point on a P_{O_2}–P_{CO_2} diagram (West, 1977). Pulmonary venous admixture was small during lung ventilation, amounting to approximately 2% of total pulmonary blood flow in *Testudo* and 6% in *Pseudemys*, but increased three- to fourfold during apnoea. The alveolar or distributional dead space probably also increased during apnoea, since the P_{O_2} gradient from alveolar gas to pulmonary venous blood usually increased (Figs. 5 and 6). The change was not large, but the slope of the regression line on the lung gas *v.* blood O_2 graph for *Testudo* is significantly greater than 1. It does appear that the lung is less efficient as a gas exchanger during these changes, mainly of vascular nature, seen during periods of apnoea.

Intraventricular shunting during intermittent breathing

If *in vitro* blood data for *Pseudemys* and *Testudo* (Burggren *et al.* 1977) are used to estimate oxygen contents from the present blood P_{O_2} measurements, then the origin of blood in each of the central arteries can readily be calculated (Table 2). The general blood distribution is as might be expected with right aorta blood being derived in the main from the left auricle and pulmonary arterial blood from the right auricle. However, simultaneous right-to-left and left-to-right shunts occur during lung ventilation and apnoea in *Pseudemys* and *Testudo*. Unfortunately, the data in Table 2 cannot be utilized to estimate the right-to-left blood shunt within the chelonian heart (i.e. the redistribution of systemic venous blood into the systemic arteries), since blood flow into each of the three systemic arteries is not equal (Shelton & Burggren, 1976), and they carry blood of different compositions (Figs. 5–8). However, the

Table 2. *The composition and origin of blood conveyed in the systemic and pulmonary arteries of* Pseudemys scripta *and* Testudo graeca *at* P_{A,O_2}*s representative of lung ventilation* (P_{A,O_2} 120 mmHg in turtle, 130 mmHg in tortoise) *and of a typical period of apnoea* (P_{A,O_2} 90 mmHg *in turtle*, 120 mmHg *in tortoise*).

		Blood origin during lung ventilation		Blood origin during apnoea	
Species	Artery	% blood from left auricle	% blood from right auricle	% blood from left auricle	% blood from right auricle
Testudo graeca	Pulmonary artery	43 (left-to-right shunt)	57	17 (left-to-right shunt)	83
	Left aorta	77	23	59	41
	Right aorta	90	10	88	12
Pseudemys scripta	Pulmonary artery	12 (left-to-right shunt)	88	11 (left-to-right shunt)	89
	Left aorta	55	45	26	74
	Right aorta	68	32	38	62

magnitude of the left-to-right shunt, which can be calculated, falls during apnoea, particularly in *Testudo* (Table 2). These data are in good agreement with estimates of left-to-right shunting in other investigations on chelonian reptiles (Steggerda & Essex, 1957; White & Ross, 1966; Shelton & Burggren, 1976), and confirm the validity of our blood gas measurements.

Separation within the ventricle of blood streams originating from the left and right auricles is clearly not complete during either apnoea or lung ventilation. There is, however, a remarkable degree of partitioning of blood when consideration is made of the arrangement and proximity of the auricular outlets and the arterial origins in the incompletely divided ventricle (see Shelton & Burggren, 1976, for gross anatomy of the chelonian ventricle). The bases of the right aorta and the brachiocephalic artery, for example, are immediately adjacent to the opening of the right auricle. During diastole and very early systole blood from the right auricle must move past the arterial bases at the anterior margin of the cavum venosum and then traverse the muscular ridges intervening between the cavum venosum and the cavum pulmonale. Yet, the right aorta and brachiocephalic artery still derive the great majority of their blood from the left auricle (Table 2), even during apnoea when right-to-left shunting is maximal. Moreover, flow of blood from the left auricle into the cavum arteriosum, then around the auricular–ventricular valves into the cavum venosum is required before ejection of pulmonary venous return into the systemic arteries can occur. Thus, during late diastole and early systole there must be a general flow of blood from the left towards the right side of the heart. Blood flow in this direction is aided during early systole by the fact that ejection into the pulmonary artery on the right side of the heart precedes systemic ejection by 50–100 msec (Steggerda & Essex, 1957; White & Ross, 1966; Shelton & Burggren, 1976). The left aorta occupies an anatomically intermediate position between the pulmonary and brachiocephalic arteries. We find, in contrast to Steggerda & Essex (1957), that the respiratory gas tensions of the blood that the left

aorta conveys suggest that it is perfused from a transitional zone in the ventricle where the interface between blood from the left and right auricles develops. Deoxygenated and oxygenated blood must come into contact within the heart, but gross admixture could be minimized if turbulent flow during ventricular filling and ejection was avoided. The significance of laminar blood flow to the separation of pulmonary and systemic venous blood in the totally undivided ventricle of amphibians has been emphasized by Shelton (1975), and within the chelonian heart the strategic positioning of the muscular ridges and the large auricular–ventricular valves might serve to promote a similar laminar flow of blood through the central cardiovascular system. Additionally, fundamental changes in the rate of spread and the pattern of electrical activities over the chelonian heart accompany intermittent breathing (Burggren, 1978). Probably by influencing the mechanical events of the heart and hence the relative positioning of the muscle ridges during systole, these pattern changes will be partially responsible for the variation in intraventricular blood admixture arising during alternating breathing and apnoea.

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