J. Exp. Biol. (1971), **55**, 47–61
With 5 text-figures
Printed in Great Britain

GAS TENSIONS IN THE LUNGS AND MAJOR BLOOD VESSELS OF THE URODELE AMPHIBIAN, *AMPHIUMA TRIDACTYLUM*

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(*Received 2 December* 1970)

INTRODUCTION

Those vertebrates which exchange their respiratory gases in both air and water present complex and interesting problems, particularly in relation to the regulation of ventilation and perfusion of the exchanging surfaces. In both lungfish and amphibia the lung is perfused via morphologically distinct pulmonary arteries and veins which form part of a partially divided double circulation. However, the incompletely divided heart in these forms is a region possessing potential for the development of extensive shunts between pulmonary and systemic circuits. The effect of such shunts would be to allow both intermixing of blood streams returning to the heart and inequalities of flow in the two circuits. Variations in the balance of flow to lungs and body, associated with breathing behaviour, have already been demonstrated in both lungfish (Johansen, Lenfant & Hanson, 1968) and amphibia (Shelton, 1970). In spite of an anatomical arrangement which would suggest very considerable mixing of blood from pulmonary and systemic inflow, selective distribution of oxygenated and deoxygenated streams has been reported by a number of workers (Delong, 1962; Johansen, 1963; Haberich, 1965; Johansen & Ditadi, 1966; Johansen *et al.* 1968). In lungfish and amphibia it was found that blood in the systemic arch was consistently more highly oxygenated than that in the pulmonary artery.

The experiments on amphibia were carried out by using either terminal blood samples to estimate oxygen levels, or other less direct methods to indicate selective distribution. Any changes that may have occurred in blood gas levels at different times were, of necessity, not monitored by these techniques. In animals which breathe in a discontinuous fashion it is likely that, between breaths, variations will occur in the oxygen and carbon dioxide levels in the body, unless some special mechanisms exist to prevent change. Some knowledge of the range of internal gas tensions over which the animal normally operates is a necessary preliminary to the analysis of ventilation: perfusion relationships in total gas exchange. Johansen & Lenfant (1968) have already demonstrated in *Protopterus* that a steady fall in arterial oxygen tension occurs from the time an air breath is taken until the following breath. There is also some indication that oxygen tensions fall and carbon dioxide tensions increase between lung ventilations in amphibia, though here the evidence is less direct. Lenfant & Johansen (1967)

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prevented lung ventilation in *Amphiuma* and *Rana catesbiana* by submerging them. They observed lower oxygen and somewhat higher carbon dioxide tensions in blood samples taken from the coeliac artery during the period of involuntary submergence. This work did not show how great the variations would be in an animal with free access to the surface and air.

In the present experiments gas tensions were monitored repeatedly in the lungs and in blood samples taken from a number of sites in the body as the experimental animal, *Amphiuma tridactylum*, moved freely in a tank of water. *Amphiuma* is exclusively aquatic and spends much of its time under water. Lung ventilation in this well-adapted diving animal is substantially less frequent than in the more terrestrial frogs and toads. The intervals between breathing movements are thus conveniently long for the type of experiment described here. It also seems likely that many other physiological adaptations associated with discontinuous ventilation, such as pulmonary vasoconstriction between breaths (Shelton, 1970) and other cardiovascular changes (Shelton & Jones, 1965), as well as the principal factors examined in this paper, may be developed in a more extreme form in *Amphiuma* than in many other amphibia.

METHODS

The experiments were carried out on 25 specimens of *Amphiuma*, weighing between 250 and 1000 g. In all cases the animals had recovered completely from any anaesthesia and were free to move in the experimental tank which was maintained at a temperature of $15 \pm 0{\cdot}5$ °C. Gas samples from the lungs or blood samples from various vessels were taken repeatedly at fairly regular time intervals throughout an experiment and analysed for oxygen and carbon dioxide. At the same time the animal was watched and a record was kept of its movements in the tank, particularly those during which it surfaced and ventilated its lungs.

Blood-vessel cannulae and lung cannulae were made from Clay-Adams P.E. 50 tubing (inside diameter 0·06 mm, outside diameter 0·10 mm) and were chronically implanted after the animals had been anaesthetized by immersion in a solution of Sandoz MS 222 at concentrations of 15 g/l. *Amphiuma* is particularly resistant to this substance but, at the very high concentrations used, anaesthesia occurred within 20–40 min and the animal remained anaesthetized for 30–60 min after being removed from the solution. The right lung, which is almost half as long again as the left one, was cannulated at its posterior end, and though the cannula was inserted into the lung for about 5 cm the samples were taken from a site which was a very considerable distance from the point of entry of the bronchus. Samples were obtained from a number of blood vessels though not more than three were cannulated in a single individual. The usual pattern followed was for the systemic circulation (and the lung) to be cannulated in all cases and one or two other blood vessels also to be sampled. The main sites for cannulation were in the systemic and pulmonary arches with the cannula tip facing upstream or downstream, in the dorsal aorta and inferior vena cava in the posterior body cavity, and in the pulmonary artery and vein as they ran over the apex of the lung. The first two sites were close to the heart and in some cases an incision had to be made in the pericardium. This was carefully sutured after the cannulation had been made. The method of cannulation permitted blood to flow past the

cannulae and in no case did it seem likely that the vessels were occluded to a significant extent. The vessels were approached via 5 cm incisions through the ventral musculature and body wall, either at the level of the anterior limbs (for pulmonary artery, systemic arch and pulmonary vein cannulations) or some 5 cm anterior to the vent (for lung, inferior vena cava and dorsal aorta cannulations). After the operation the incisions were closed with Clay-Adams 9 mm wound clips. All the blood cannulae were filled with heparinized saline (250 i.u./ml) to prevent clotting. Approximately 1·0 ml of the heparinized saline/kg was injected immediately after the operation.

Blood was sampled by allowing it to flow, using the animals' blood pressure, through the cannulae into two Radiometer thermostatted cells, one containing an oxygen electrode and the other a carbon dioxide electrode. The electrodes were connected to a Radiometer pH meter 27, fitted with a Gas Monitor PHA 927. The output of the pH meter was recorded on a Gilson polygraph. About 200 μl of blood were needed for a determination of oxygen and carbon dioxide tensions. After the determinations had been made (3–5 min) the blood was returned to the circulation by increasing the pressure on the sample chambers. A negligible amount of blood was lost and the total blood volume was not seriously disturbed. The oxygen electrode was calibrated using an oxygen-free sodium sulphite/borax solution and air-equilibrated saline. Salines equilibrated with gas mixtures containing 1 and 7% carbon dioxide were used to calibrate the carbon dioxide electrode. Electrodes were calibrated at the beginning and end of an experiment or, in the case of prolonged runs, at approximately 2 hourly intervals.

Alveolar gas samples up to 0·2 ml in volume were analysed for oxygen, nitrogen and carbon dioxide in a Varian Aerograph gas chromatograph Series 200. The separation columns used in the chromatograph were Silica-gel (screen size 42/60) and Molecular sieve 5A (screen size 42/60). The columns were arranged in series with the thermal conductivity detector. The total amount of time required for the analysis of one sample was 6 min. Gas samples were taken from the lung in a 0·25 ml Hamilton gas-tight syringe and injected into the gas chromatograph. The equipment was calibrated by injecting gas mixtures of known composition.

When an arterial cannula was not being used to obtain a blood sample for gas analysis, it was disconnected from the thermostatted cells and coupled to a Statham 23 AA pressure transducer. Arterial blood pressures were thus monitored intermittently throughout an experiment and were recorded on the Gilson polygraph.

RESULTS

Oxygen tensions

The lung and the systemic circulation were cannulated in all animals in order to give an adequate basis for comparison of data from other sites. As a result more data are available for these regions than for the others described. The oxygen tensions in the lungs varied over a wide range as *Amphiuma* remained submerged and periodically surfaced to breathe (Figs. 1–3, 5). The average period between breaths in 11 of the experimental animals was 44·9 min (± 3·9 S.E.). The standard deviation was 23·2 min indicating that duration of the breathing interval varied considerably between individuals. Even in a single *Amphiuma* breathing was not a markedly regular event, long

intervals sometimes alternating with short ones. Another pattern often seen was one in which an animal surfaced to breathe after a substantial interval and then repeated the emergence and breathing after short intervals, up to 5 or 10 min in duration, even though the first breathing period had substantially increased oxygen tensions in lungs and blood (Fig. 2).

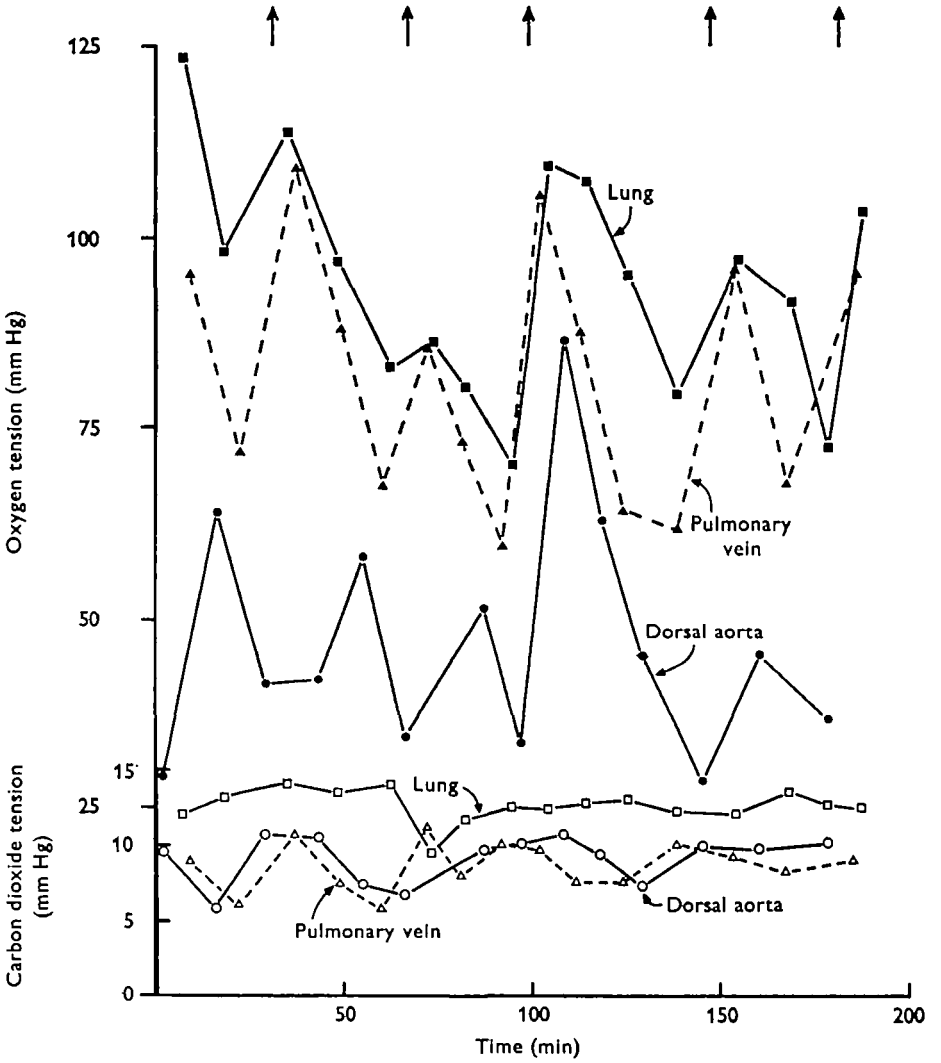

Fig. 1. Changes in oxygen (closed symbols) and carbon dioxide (open symbols) tensions in the lung, dorsal aorta and pulmonary vein of a 250 g *Amphiuma* during five breathing–diving cycles. Vertical arrows indicate times at which the animal surfaced and ventilated its lungs.

During the breathing interval oxygen tension in the lung gases fell from a mean level of 95·8 mmHg (± 5·3 S.E.) immediately after a breath to 47·1 mmHg (± 3·0 S.E.) immediately before the next one. Again there was considerable variation in the oxygen tensions measured at these times, as the standard deviations (22·2 and 12·7 respectively) show. This was true even in a single animal since breathing did not always increase oxygen tensions to the same level as the previous breathing series nor

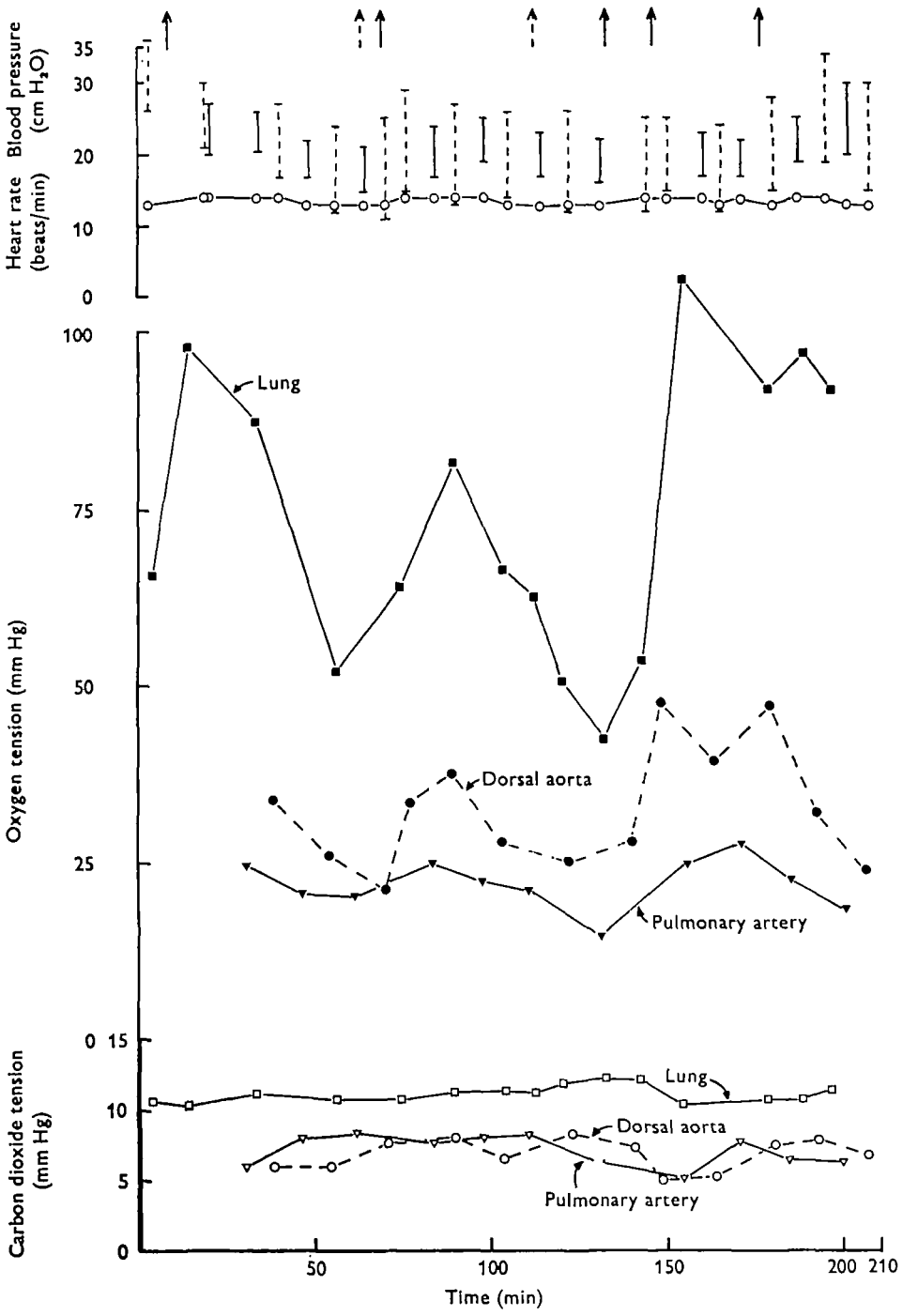

Fig. 2. Changes in oxygen (closed symbols) and carbon dioxide (open symbols) tensions in the lung, dorsal aorta and pulmonary artery of a 650 g *Amphiuma* during four breathing–diving cycles. Heart rate and blood pressures are also plotted. The systolic and diastolic pressures are joined by a dashed line when they were measured in the pulmonary artery and by a continuous line when measured in the dorsal aorta. Vertical arrows indicate times at which the animal surfaced; if it also ventilated its lungs the arrows are in continuous line, if it submerged without ventilation the arrows are dashed.

was breathing triggered when lung oxygen tensions reached some critical low level (Figs. 1, 2).

No obvious differences could be found between the arterial blood samples taken from the systemic arch or from the dorsal aorta in the posterior region of the body. The rapid rise in lung oxygen tensions following a breath resulted in an almost simultaneous rise in the gas tension of blood samples taken from these arteries (mean 56·4 mmHg ± 3·9 S.E.). Thereafter the tension declined slowly to a minimum just before the next breath (mean 21·4 mmHg ± 2·5 S.E.). As with the lung tensions, the standard deviations (18·5 and 11·8 respectively) show that there was considerable variation between individuals (Figs. 1–3, 5) and in the high and low levels reached at the beginning and end of different dives in a single *Amphiuma* (see, for example, Fig. 1).

As might be expected in an animal with a substantial central shunt between oxygenated and deoxygenated blood, the oxygen tensions in the pulmonary vein were higher than those in the major arteries of the systemic circulation. In fact, as Fig. 1 shows, the tensions were very much closer to those measured in the lungs than they were to those in the dorsal aorta, especially when the samples were taken just after a breath. The oxygen gradient from lung to blood was then very small but as the duration of a dive increased, following a breath, so the gradient became progressively larger. The change in gradient was seen most clearly in the animal from which Fig. 1 was taken; in the other two animals examined the gradient increased during a dive but in a somewhat less pronounced fashion. The effect of this change in gradient was that the oxygen tensions in the pulmonary vein moved through a wider range than those in the lungs during each breathing cycle.

In spite of the central shunt complete mixing of venous and arterial blood streams did not occur in the heart. Oxygen tension of the blood in the pulmonary artery was consistently lower than that in the dorsal aorta (Figs. 2, 5). Because oxygen tensions in the pulmonary artery fluctuated over a much smaller range than those in the dorsal aorta, the difference was greatest immediately after a breath and became progressively less marked during the time that the animal remained submerged. The oscillations in oxygen tension due to periodic breaths were very much more damped in this blood vessel than in the others so far considered, and in a few records (e.g. Fig. 5) there is some evidence that the peak oxygen tensions were reached rather later.

Oxygen tensions in blood taken from the interior vena cava were also lower than those in the dorsal aorta (Fig. 3). Relatively few determinations were made on vena cava blood and it is difficult to be precise about the temporal relationships of the tension changes, though there can be no doubt that fluctuations occurred. These followed the form already seen, reaching a peak sometimes after a breath and falling gradually thereafter. The oscillations were of smaller magnitude than those in blood from the dorsal aorta.

Oxygen tension relationships

In order to compare the oxygen tensions found in gas and blood samples from different sites, results from nine different animals have been combined to produce the graphs of Fig. 4. In these, oxygen tensions in the various blood samples are plotted against oxygen tensions in the lung gases over the whole range measured during several breathing cycles. Regression lines have been calculated for the four groups of data from different blood vessels. A measure of the lung–blood oxygen difference at any

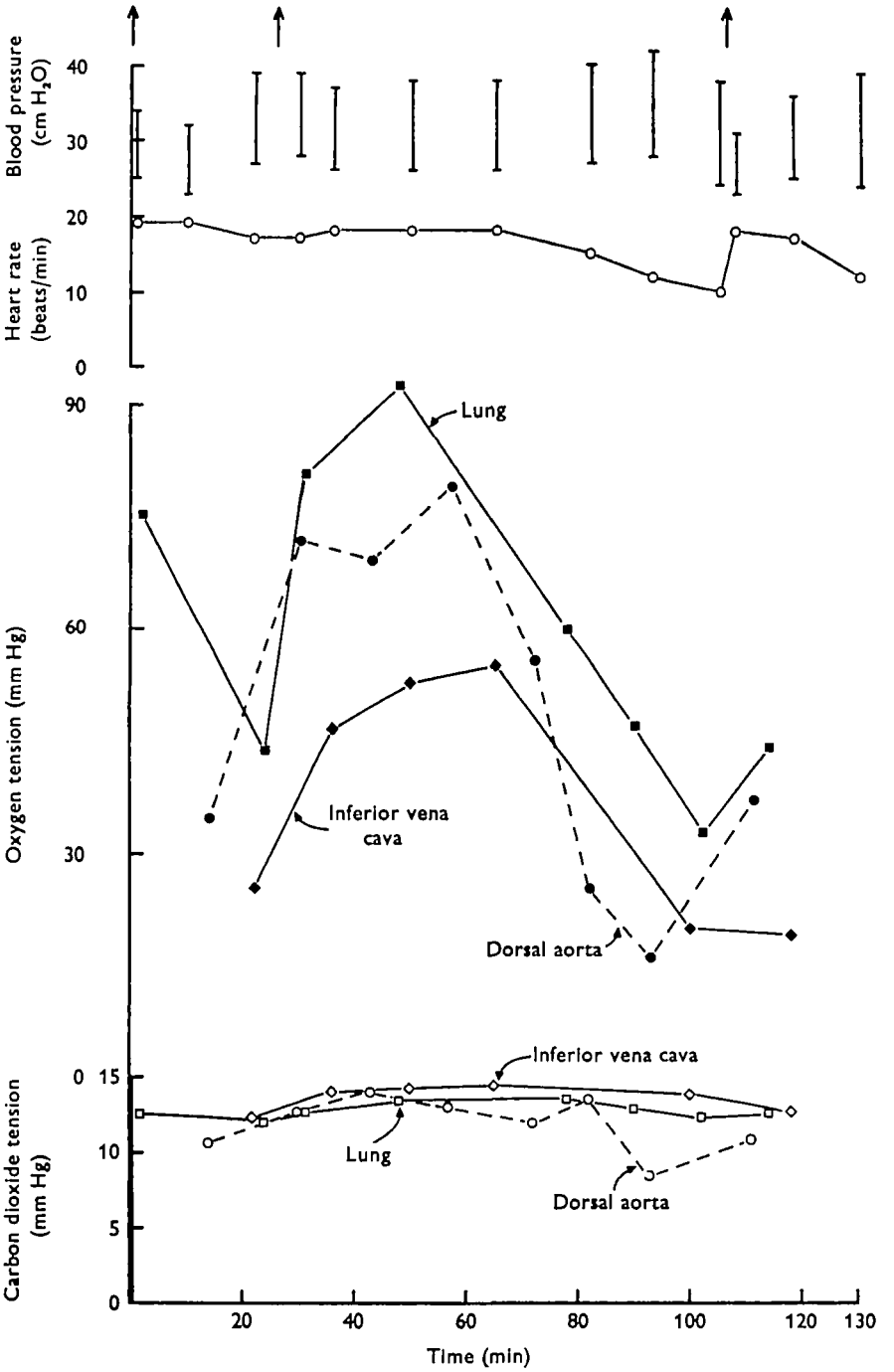

Fig. 3. Changes in oxygen (closed symbols) and carbon dioxide (open symbols) tensions in the lung, dorsal aorta and inferior vena cava of a 515 g *Amphiuma* during two breathing–diving cycles. Heart rate and systolic and diastolic blood pressures in the dorsal aorta are also plotted. Vertical arrows indicate times when the animal surfaced and ventilated its lungs.

lung oxygen tension is thus given by the distance between the lung (45°) line and the blood regression line. The different blood oxygen lines can be compared on the same basis and the dorsal aorta regression line is plotted on each graph for this purpose. Since oxygen tensions in both the lung and dorsal aorta were measured in all cases, either could have been made the basis of comparison and used as the variable plotted on the abscissa in the graphs. In fact no significant difference emerged with either treatment except in the case of the inferior vena cava results described below.

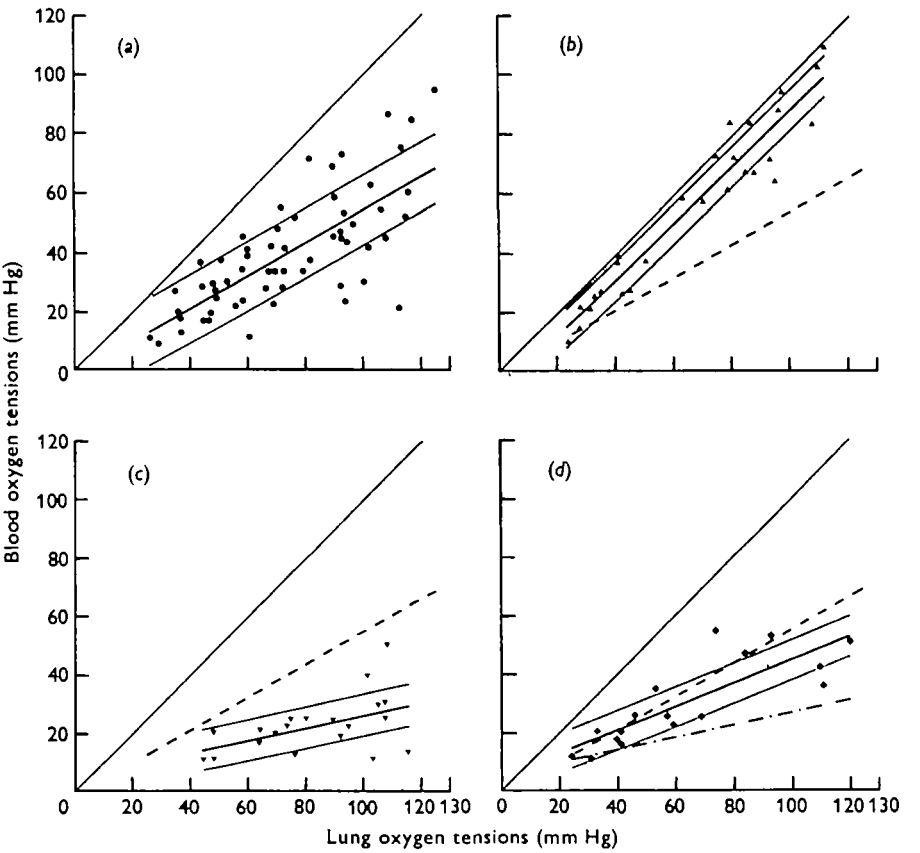

Fig. 4. Relationship between blood oxygen tensions and lung oxygen tensions, showing (continuous lines) regressions and standard deviations calculated from the data plotted. The distance between the 45° line and the regression line gives a measure of the average lung–blood vessel difference at any lung oxygen tension. The regression line for dorsal aorta data shown in (*a*) is also plotted in (*b–d*) as a dashed line. (*a*) Dorsal aorta data. Regression line slope 0·56, intercept −1·8. (*b*) Pulmonary vein data. Regression line slope 0·96, intercept −7·7. (*c*) Pulmonary artery data. Regression line slope 0·21, intercept +5·2. (*d*) Inferior vena cava data. Regression line slope 0·40, intercept +5·0. If dorsal aorta–inferior vena cava differences are plotted using the dashed dorsal aorta line as the reference, then a new inferior vena cava line can be calculated. This is shown by the dashed–dotted line, slope 0·21, intercept +6·1.

The pulmonary artery, pulmonary vein, and inferior vena cava plots are derived from the results of experiments on three animals in each case. The small gradient between lung and pulmonary vein over the whole range of oxygen tensions is clearly seen (Fig. 4*b*). What is lost in this combined plot, because of the variety of operating ranges encountered in the three animals, is the increase in gradient as tensions fall

during a dive (Fig. 1). As expected, the lines for the dorsal aorta (Fig. 4*a*) and pulmonary artery (Fig. 4*c*) lie below that for the pulmonary vein and their slopes are, respectively, some $\frac{2}{3}$ and $\frac{1}{6}$ of the pulmonary vein slope. The regression line derived from the data for the lung–inferior vena cava relationship is below the calculated dorsal aorta line, as one might expect, but not markedly so (Fig. 4*d*). In fact, as plotted directly it lies above the pulmonary artery line and has a steeper slope, both of which are extremely unlikely if it is truly representative of mixed venous blood as it enters the heart. There are two possible explanations of this very high venous oxygen tension. The first is that the sample taken from the posterior end of the body cavity is not representative of mixed venous blood but contains, for example, a high percentage of blood returning from the skin. The second, and probably more important, factor is that all three animals in which inferior vena cava determinations were made showed very small lung-dorsal aorta differences so that the dorsal aorta line for these animals alone is significantly steeper than the line derived from nine animals as shown. If the actual dorsal aorta–inferior vena cava differences are plotted on the graph in relation to the dorsal aorta regression line as drawn, a new inferior vena cava line of much smaller slope can be derived (Fig. 4*d*). This is almost identical in slope and position to the pulmonary artery line.

Carbon dioxide tensions

The carbon dioxide tensions recorded in lung and blood samples fell into the same general range as those previously reported for amphibia and lungfish (Johansen, Lenfant & Grigg, 1967; Lenfant & Johansen, 1967; Lenfant & Johansen, 1968). They are low, as might be expected in an aquatic animal intermittently breathing air, but not as low as in entirely water-breathing fish. In the lung the mean carbon dioxide tension found in all samples except those taken immediately after a breathing period was 14·9 mmHg (±0·5 S.E.). If the first samples taken after breathing are pooled a slightly lower mean value is found (14·6 ± 0·6) but this does not differ significantly from the overall figure. In some individuals larger falls in carbon dioxide level (up to 5 mmHg) were found immediately after breathing, though changes larger than 2 or 3 mmHg were not common. Samples had to be taken soon after a breathing period in order to detect significant changes because the prebreath level was reached within 2 or 3 min and thereafter fluctuations were small (Figs. 1–3). In only one out of 22 experiments in which the lung carbon dioxide was measured did the levels increase to any extent as the animal remained submerged.

Carbon dioxide tensions in blood from the major vessels were usually slightly lower than those measured in the lungs (Figs. 1, 2), though this was not invariably the case (Fig. 3). The mean tension found in the dorsal aorta samples taken before a breath was 11·5 mmHg (±0·9 S.E.), while that in samples immediately before a breath was 10·9 (±0·8 S.E.). Again the two values do not differ significantly. Little consistent difference could be seen between the tensions in the dorsal aorta and those found in the other blood vessels. Even in a single individual fluctuations of some 3 or 4 mmHg would occur over roughly the same range in all the blood vessels sampled (Figs. 1, 2). Some of these fluctuations could be related to lung ventilation, others could not. As in the case of the lung, a slow increase in carbon dioxide tensions throughout the full period of a dive was not seen in any of the blood vessels.

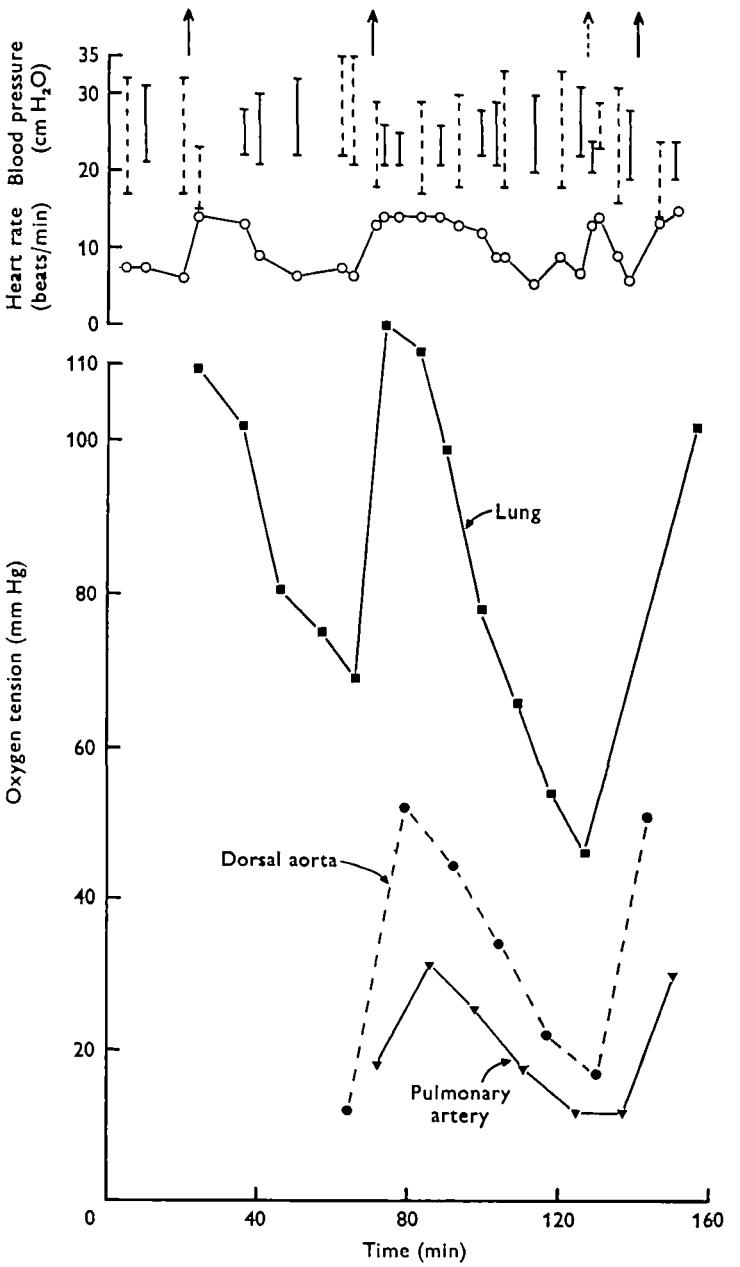

Fig. 5. Changes in oxygen tensions in the lung, dorsal aorta and pulmonary artery of a 440 g *Amphiuma* during two breathing–diving cycles. The heart rate and blood pressures are also plotted. The systolic and diastolic pressures are joined by a dashed line when they were measured in the pulmonary artery and a continuous line when measured in the dorsal aorta. Vertical arrows indicate times at which the animal surfaced; if it also ventilated its lungs the arrows are in continuous line, if it submerged without breathing the arrows are dashed.

Heart rate and blood pressure

The heart rate was determined in 14 of the animals and the mean rate was 13·6 beats/min (± 0·4 S.E.). In most, the rate was higher than this just after a series of breathing movements and then the heart slowed steadily to a lower value during a dive (Figs. 3, 5). A few animals showed very little evidence of this diving bradycardia (Fig. 2).

Blood pressures recorded in systemic and pulmonary arches reached similar systolic values but fell to very different diastolic levels, the pulmonary diastolic pressure always being the lower of the two. This pattern is very similar to that recorded by Shelton & Jones (1968) in anuran amphibia. They suggested that it was associated with the presence of a spiral valve in the conus and confirmed that it was absent in the salamandar which does not have the valve so well developed. Though *Amphiuma* is a urodele, the conus does possess a very large spiral valve. The difference in pulse sizes can be seen in Figs. 2 and 5. In these figures the systemic pressures were measured at the posterior end of the dorsal aorta and both systolic and diastolic levels were somewhat lower than they would have been in records obtained from more central locations. Determination of the blood pressures in the two arteries as they emerged from the heart showed that the systolic pressures were almost identical.

Systolic and diastolic pressures in both systemic and pulmonary vessels usually decreased when the animal ventilated its lungs (Fig. 5). During this period there was also a marked fall in pulse pressures in both vessels. To some extent this can be explained in terms of the accompanying increased heart rate, though there may be other changes involved. The factors producing the increased heart rate and fall in blood pressure are obviously complex because similar changes occurred when the animal surfaced but did not breathe (Fig. 5). After such an excursion to the surface the low heart rate, high pressures and large pulse were restored much more rapidly than they were after lung ventilation. Clearly, the act of breathing has some influence on the responses seen though it is not the only factor involved. In a few animals the changes in blood pressure during the breathing–diving cycle were quite small, as they were for the heart rate (Fig. 2).

DISCUSSION

The movements of the buccal floor and the consequent alterations in buccal volume made when *Amphiuma* comes to the water surface, result in a substantial exchange between lung gas and the outside air. The exchange occurs entirely through the nares which usually are the only part of the head raised above the water surface. Progressively falling oxygen tensions in the lungs during a dive establishes that this gas is slowly removed by the animal. Equally the constant carbon dioxide tensions suggest that the lungs are not important in removing this gas but that some other route, most probably the skin, is of much greater significance. Taken together these considerations lead to the conclusion that the lungs gradually decrease in volume during a dive. We have confirmed that the total volume of the animal does decrease by roughly 5–6 ml/kg/45 min dive, and it seems reasonable to ascribe the whole of the decrease to fall in lung volume. Since the animal does not change in volume in the long term, it must be concluded that a series of buccal movements made during a single emergence both removes gas from the lungs and replaces it with a slightly greater quantity of air.

The precise timing of the events of ventilation was not studied in the present work but the overall result can be seen from the oxygen tension data. Using the average figures a final lung oxygen tension of 96 mmHg after breathing would be produced by a mixture of 53% air and 47% of the prebreath lung gases with an oxygen tension of 47 mmHg. Our measurements of lung volume in *Amphiuma* indicate that it represents some 6–7% of the total body volume so that the average breathing cycle would result in an intake of approximately 37 ml air in a kilogram animal. The figures on volume change suggest that, on average, only 31 ml of the original lung gases would be expired. There are thus oscillations of a substantial nature both in lung volume and lung oxygen concentration, closely related to the breathing rhythm.

Changes in oxygen tension, similar to those seen in the lung are also found in all parts of the circulatory system, being largest in the pulmonary vein and becoming progressively smaller in dorsal aorta, pulmonary artery, and inferior vena cava. The large oscillations seen in the pulmonary vein occur because the oxygen tension difference between lung and vein increases during the course of a dive. Experiments in which volume was measured in submerged *Amphiuma* showed that volume change occurred more rapidly at the beginning of a dive than at the end, indicating that the rate of oxygen removal was decreasing in spite of an increased gradient. It therefore seems likely that the characteristics of the pathway between alveoli and red blood cells are changing to produce a decrease in transfer factor ($T_{L_{O_2}}$) of the whole lung:

$$T_{L_{O_2}} = \frac{\dot{V}_{L_{O_2}}}{\Delta P_{L_{O_2}}},$$

where $\dot{V}_{L_{O_2}}$ is the rate of oxygen transfer from alveolar air to blood and $\Delta P_{L_{O_2}}$ is the mean oxygen gradient across the respiratory epithelium. Although it is difficult to be sure that changes in oxygen tension difference between alveolar air and pulmonary vein as actually sampled are indicative of similar changes in $\Delta P_{L_{O_2}}$, the suggested decrease in transfer factor during submergence seems to be substantiated by other observations. Experiments on other amphibia have shown that vasoconstriction occurs in the lungs in periods between ventilation (Shelton, 1970). Pulmonary vasoconstriction, producing both a longer diffusion pathway and a change in the perfusion pattern could well be the cause of decreased transfer factor in *Amphiuma* during a dive. The blood pressure data are consistent with this suggestion, since there is almost invariably a considerable fall in mean blood pressure when the animal breathes and a steady increase during a dive. The fall in pressure occurs at the same time as the heart recovers from a bradycardia so that the output, if it is changing at all, is probably rising (Shelton & Jones, 1965). Vasodilation of some part of the periphery must be responsible. Because pulmonary and systemic circuits arise from the single pressure source, vasodilation need not occur uniformly in both circuits for the systolic pressures, at least, to be similarly affected. The pressure recordings, though indicating that vasomotor changes occur, provide no unequivocal information as to their site.

Blood from the pulmonary vein is obviously contaminated with that from the body as it passes through the heart because oxygen tension is much lower in the dorsal aorta than it is in the pulmonary vein. If there is selective vasoconstriction in the lungs during a dive, then the extent of this contamination should vary, since blood from the

body would make a greater contribution to the blood leaving via the dorsal aorta at these times. In an extreme case, for example, a total block in lung perfusion would result in all blood in the systemic arteries being derived from the right auricle. With progressive pulmonary vasodilation more and more of the blood in the dorsal aorta would be derived from the left auricle. The relative position of the regression lines calculated from the pooled data suggests that contamination varies in the way predicted. Some caution must be exercised in using the pooled data, particularly in the case of that from the inferior vena cava where there is uncertainty that the samples were representative of mixed venous blood such as would be obtained from the right auricle. Another difficulty arises because of the difference in position of the regression line depending on whether the lung–inferior vena cava differences or the dorsal aorta–inferior vena cava differences are used for the plot. The latter, which produce the lower line in Fig. 4*d*, are obviously more appropriate in the present case where differences in blood oxygen tensions are being considered. Using blood data for *Amphiuma* presented by Lenfant & Johansen (1967) to calculate approximate oxygen contents at different points on the pulmonary vein, dorsal aorta, and inferior vena cava regression lines, it appears that the percentage of dorsal aorta blood derived from the pulmonary vein varies from 80% at the upper end to 40% at the lower end of the oxygen tension range.

The results so far discussed do not require selective distribution during the movement of blood through the heart. They could occur simply through gross variation in the relationship between pulmonary and systemic flow rates. However, the fact that oxygen tensions in the pulmonary artery are always lower than those in the dorsal aorta indicates that selective distribution is maintained at all times. The similarity between oxygen tensions in the pulmonary artery and in the inferior vena cava, at all levels, leads to the conclusion that there is little contamination from the pulmonary vein as blood passes from the right auricle to the pulmonary artery. In general this work suggests that there is a marked selective distribution of blood just after the animal has breathed with more shunting from right to left than in the reverse direction. As a dive progresses and pulmonary vasoconstriction occurs, the degree of right-to-left shunting increases in a marked way so that ultimately the dorsal aorta receives the major part of its blood from the right auricle. The whole problem of selective distribution and the extent of shunting in the amphibian ventricle and conus is a controversial one. Several workers (de Graaf, 1957; Johansen, 1963; Johansen & Ditadi, 1966) have suggested that, in some anurans and in *Amphiuma*, the major shunt is from left to right. This pattern would demand a higher rate of blood flow to the lungs than to the body, and direct measurements of flow indicate that this is not the case in anurans at least (Haberich, 1965; Shelton, 1970; Johansen, Lenfant & Hanson, 1970). These measurements are not inconsistent with the hypothesis presented here of a major right-to-left shunt of changing magnitude. It is perhaps the variable nature of the shunt which has given rise to some of the conflict in previous results and interpretations.

The small fluctuations in carbon dioxide tension in both lung and blood samples can be correlated with the known importance of the skin as a site for carbon dioxide removal in amphibia (Lenfant, Johansen & Hanson, 1970; Whitford & Hutchison, 1963). In the present experiments such fluctuations as did occur could not always be

related to the breathing–diving cycle. In some cases a fall of carbon dioxide level was seen in samples taken very soon after a breath, but in no case was a gradual increase seen during the course of a prolonged dive. The results suggest that, at 15° C at least, *Amphiuma* can maintain constant internal levels of carbon dioxide by removing the gas almost entirely through the skin.

This is not to say that lung ventilation does not have a small part to play in the removal of carbon dioxide. If, as suggested above, an average breathing series results in the lungs containing a mixture of 53% air and 47% of the preventilation gas, it is clear that the partial pressure of carbon dioxide must fall to slightly less than half its original level. The volume of carbon dioxide in the lungs of a kilogram animal would fall from approximately 1·4 to 0·7 ml. This small quantity of gas would be replaced rapidly from the very considerable carbon dioxide pool held in the tissues and blood stream. Using realistic values for blood volume and carbon dioxide combining power, it is clear that the lung carbon dioxide could be restored to its original value by the passage of much less than the total blood volume through the lungs, at a time when lung blood flow is at a maximum. The time involved for such restitution would not be more than 1 min and the overall effect on carbon dioxide tensions in the blood and tissues would be scarcely measurable.

A feature of the results that was commonly seen was that slightly higher carbon dioxide tensions were measured in the lungs than in the blood stream. This is difficult to account for adequately other than by a consistent error in calibration of the two instruments used for the determinations. If equilibrium between blood and carbon dioxide and lung carbon dioxide was reached early when the transfer factor was large, following which the lung gradually decreased in volume and the transfer factor also fell substantially, it would be possible to produce a reversed gradient. This would be a relatively minor effect, however, and would not satisfactorily explain the observed differences.

The variability in the duration of the dives and in the levels of oxygen in lungs and blood both before and after breathing suggests that none of these factors acts in a simple way in determining when and for how long a series of lung ventilations is produced. The changes in lung volume, being related in the main to oxygen tension within the lung, are equally variable and again could not be considered as a simple trigger which would initiate or stop a series of breathing movements. Furthermore it is unlikely that any control mechanism which may exist to help maintain the relatively constant levels of carbon dioxide could be involved in the control of breathing movements since these are of little significance in the removal of the gas. The problem of control is thus a very considerable one both in *Amphiuma* and in other animals exchanging gases in air and water (Johansen & Lenfant, 1968). These animals tolerate large variations in interval oxygen tension though they must regulate in some way within this broad range. Further work is in progress on the nature of the regulating mechanisms.

SUMMARY

1. Oxygen and carbon dioxide tensions were determined in the lungs and in blood from the dorsal aorta, pulmonary vein, pulmonary artery and inferior vena cava in the intact, free swimming, *Amphiuma*. At 15° C this animal was submerged for a large

part of the time and surfaced briefly to breathe at variable time intervals, the mean period being 45 min.

2. Oxygen tensions in the lungs and in all blood vessels oscillated with the breathing cycles, falling gradually during the period of submersion and rising rapidly after the animal breathed. The absolute level of oxygen tension did not appear to constitute the effective signal beginning or ending a series of breathing movements.

3. A small oxygen gradient existed between lungs and blood in the pulmonary vein immediately after a breath. The gradient increased in size as an animal remained submerged due, it is suggested, to lung vasoconstriction increasing the transfer factor.

4. Blood in the dorsal aorta had a lower oxygen tension than that in the pulmonary vein. A right-to-left shunt occurred as blood moved through the heart. The degree of shunting increased as the animal remained submerged and pulmonary vasoconstriction occurred. Left-to-right shunt was relatively insignificant since oxygen tensions in the inferior vena cava and pulmonary artery were very similar.

5. Carbon dioxide tensions were relatively constant during the breathing–diving cycle since *Amphiuma* removed almost all of this gas through the skin.

The investigation was supported by grants from the Fisheries Research Board of Canada, the National Research Council of Canada, and the British Columbia Heart Foundation, to all of whom we express our thanks.

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