J. exp. Biol. **137**, 53–74 (1988)
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BLOOD FLOW PATTERNS IN THE SALAMANDER, *AMBYSTOMA TIGRINUM*, BEFORE, DURING AND AFTER METAMORPHOSIS

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Accepted 9 February 1988

Summary

The patterns of blood flow through the complex circulation of the tiger salamander, *Ambystoma tigrinum*, were investigated during aquatic normoxia and hypoxia by application of the microsphere technique. The distribution of differently labelled microspheres injected into the bloodstream towards the left and right atria, respectively, was used to evaluate the role of the ductus arteriosus in lung perfusion before, during and after metamorphosis, as well as the general contribution of right and left atrial outputs to the blood flow in gills and lungs in neotenic and postmetamorphic animals.

The distribution patterns of radioactive microspheres among pulmonary, branchial and systemic tissues indicated that the ductus arteriosus is the major pulmonary perfusion pathway in neotenic and metamorphosing animals, whereas after metamorphosis the main perfusion pathway is down the entire length of the pulmonary artery. In neotenes, the ductus arteriosus becomes even more important during aquatic hypoxia. The anterior branchial arches receive blood richer in pulmonary venous blood than the posterior arches. Approximately 26 % of left atrial output and 36 % of right atrial output perfuses the branchial respiratory lamellae during normoxia in neotenes. Severe aquatic hypoxia appears to increase the fraction of cardiac output flowing to the lung and decrease the fraction flowing into the first branchial arch in neotenes. This decrease into the first arch may facilitate lung perfusion and also reduce branchial O_2 loss. In postmetamorphic animals, approximately 55 % of right atrial output and 32 % of left atrial output is directed to the lungs. The flow patterns in postmetamorphic animals remain unaffected by aquatic hypoxia.

Introduction

The neotenic tiger salamander, *Ambystoma tigrinum*, is completely aquatic and utilizes skin, gills and lungs for gas exchange. The skin and gills respire with the

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Key words: amphibia, blood flow, cardiovascular shunting, ductus arteriosus, hypoxia, gill, lung, metamorphosis, pulmonary artery.

water environment, whereas the lungs are periodically ventilated with air. Pulmonary respiration accounts for approximately 45 % of total O_2 uptake at 23 °C under normoxic conditions (Heath, 1976), but its importance increases with temperature (Whitford & Sherman, 1968) and in hypoxic water (Heath, 1976). The relative importance of skin and gills in gas exchange, however, is unknown.

During metamorphosis, the gills disappear leaving only the skin and lungs for gas exchange. Pulmonary respiration after metamorphosis accounts for approximately 65 % of total O_2 uptake at 23 °C during normoxic conditions. This value rises to approximately 87 % in hypoxic water ($1{\cdot}5\,mg\,O_2\,l^{-1}$) (Heath, 1976).

Although gas exchange patterns have been studied, *in vivo* measurements of respiratory organ blood flow have never been performed. One reason for this may be the extremely complex anatomy of the central vascular system. In the heart, which is typical of amphibian hearts, systemic venous blood enters the sinus venosus which empties into the right atrium. Pulmonary venous blood flows directly into the left atrium. Both atria empty into a common ventricle, from which blood flows through the conus arteriosus and then into the bulbus arteriosus giving rise to four pairs of aortic arches (Gilmore & Figge, 1929). In other amphibians, intracardiac flow patterns cause blood entering the posterior aortic arches to be richer in systemic venous blood than blood entering the anterior arches (Johansen, 1963; Johansen & Ditadi, 1966; Meyers, Moalli, Jackson & Millard, 1979; Tazawa, Mochizuki & Piiper, 1979).

In neotenic tiger salamanders, the first three pairs of aortic arches are afferent branchial arteries, each vessel perfusing a gill, the fourth represents the pulmonary artery (Fig. 1). Within each gill there are two major perfusion pathways. One is a respiratory route from the afferent branchial artery through the respiratory lamellae and then into the efferent branchial artery. The other is a shunt directly connecting the afferent and efferent branchial arteries at the base of the gill. The efferent arteries from each of the gills unite to form the dorsal aorta. Both the internal and external carotid arteries emerge from the first branchial arch. Accordingly, the head receives most of its blood from the first arch. The ductus arteriosus connects the efferent artery of the third gill with the pulmonary artery. Proximal to the ductus arteriosus, the pulmonary artery forms a vascular plexus. This vascular organization provides two parallel pathways for lung perfusion: (1) a direct route from the heart down the entire pulmonary artery, and (2) an indirect route from the heart to the third gill, and then from the third efferent branchial artery, through the ductus arteriosus and down the distal half of the pulmonary artery.

In the neotenic animal, the ductus arteriosus is visibly larger than the pulmonary artery proximal to the ductus arteriosus. This suggests that more blood reaches the lung *via* the third gill and ductus arteriosus than through the proximal segment of the pulmonary artery. During metamorphosis, however, the gills disappear, the branchial shunt vessels are incorporated into the tubular aortic arches, the proximal segment of the pulmonary artery enlarges, the pulmonary arterial plexus disappears and the third branchial arch and ductus arteriosus diminish in size

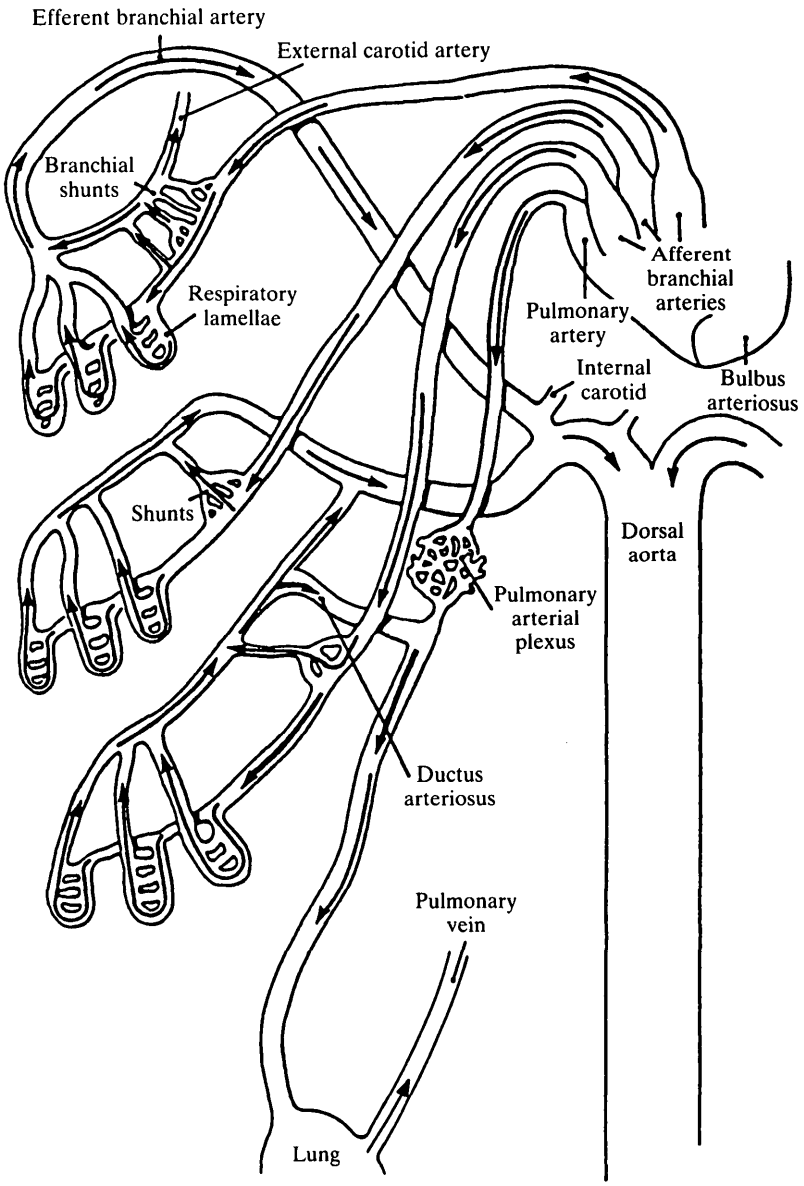

Fig. 1. Central vascular organization of the neotene tiger salamander, *Ambystoma tigrinum*.

(Figge, 1934; Gilmore & Figge, 1929; Fig. 2). These morphological changes suggest that during metamorphosis the primary perfusion pathway to the lung shifts from the ductus arteriosus to the more direct route down the entire pulmonary artery. However, the relative importance of the two pathways is not known since blood flow through the two routes has never been measured.

The third respiratory organ, the skin, receives blood from the systemic arterial system, and cutaneous venous blood drains into systemic veins. Blood flow to the skin, however, has not been addressed by this study.

The major goal of this study was to describe blood flow patterns to the gills and lungs through this complex circulation. Two specific aims were pursued. First, the relative importance of the two pulmonary perfusion pathways was assessed in neotenic, metamorphosing and postmetamorphic animals under two environmental conditions: normoxia and aquatic hypoxia. Aquatic hypoxia was expected to increase pulmonary blood flow. Second, the fraction of cardiac output flowing to the respiratory sections of the gills and to the lungs was evaluated. Both neotenic and postmetamorphic animals were studied during the two environmental conditions of normoxia and aquatic hypoxia.

To assess flow patterns to the lung, one of the pulmonary perfusion pathways was ligated before radiolabelled microspheres (MS) were infused into the systemic venous system. MS trapping in the lungs was used as an index of pulmonary perfusion. The distribution of cardiac output to the gills and lungs was investigated by simultaneous injections of differently labelled MS into the systemic and pulmonary venous systems. MS trapping in the gills, the lungs, the head and the remainder of the animal was used to assess the distribution of right and left atrial outputs into the different aortic arches, the branchial respiratory sections and the lungs.

Materials and methods

Animals

Neotenic ($N = 39$) and postmetamorphic ($N = 23$) tiger salamanders (body mass $75{\cdot}0 \pm 11{\cdot}9$ and $24{\cdot}8 \pm 8{\cdot}2$ g, respectively, $\bar{x} \pm$ S.D.) were obtained from Charles D. Sullivan Co. Inc. (Nashville, TN, USA) and maintained at 15 °C for at least 1 week prior to experimentation. Metamorphosing animals (body mass $49{\cdot}2 \pm 4{\cdot}0$ g, $N = 10$) were obtained by subcutaneous injection of neotenes with thyroxine (100 mg/animal, dissolved in 0·5 ml of 0·1 mol l^{-1} NaOH) into the back (Hackford, Gillies, Eastwood & Goldblatt, 1978). After thyroxine treatment the animals were maintained at 15 °C for 10 days and then used for experiments. Gill regression started within a few days, and after the experiment (11 days after thyroxine administration) gill mass had decreased by approximately 75 %. Net gill mass of the neotenes used was $1{\cdot}62 \pm 0{\cdot}4$ g ($N = 39$) and that of the metamorphosing animals was $0{\cdot}40 \pm 0{\cdot}06$ g, $N = 10$. In *Ambystoma tigrinum*, thyroid-releasing-factor-induced metamorphosis results in a 75 % reduction in gill length during approximately the same fraction of the total metamorphic period (Norris, Jones & Cohen, 1973). This degree of gill resorption has also been used to defined late metamorphic stages in *Ambystoma mexicanum* (Sawin *et al.* 1978).

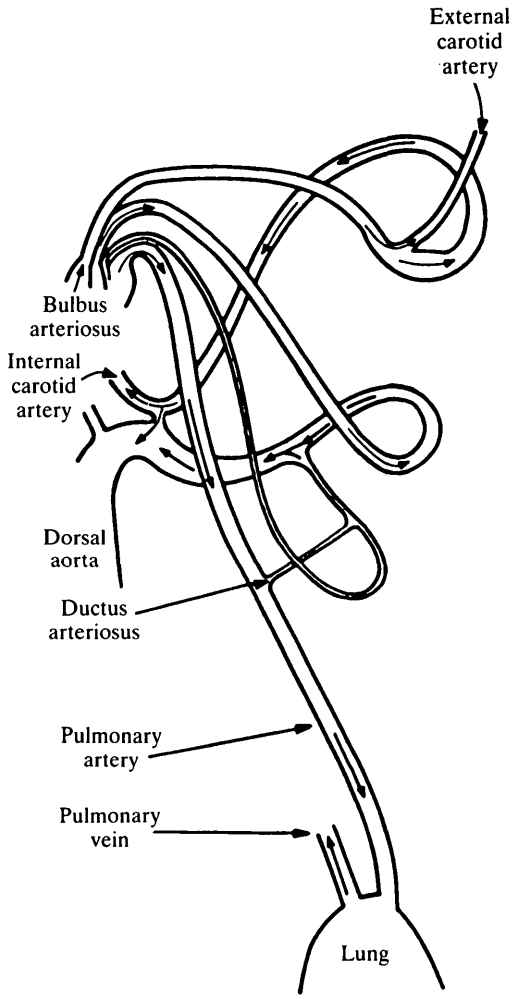

Fig. 2. Central vascular system of metamorphosed tiger salamanders, *Ambystoma tigrinum*.

Procedure

Experiments assessing the two pulmonary perfusion pathways

Animals were anaesthetized by immersion in MS-222 (Sigma, 0·1 % solution). After loss of reactivity the animals were removed from the anaesthetic bath and placed on the operating table, the skin being kept moist with saline-soaked tissues. A cannula (PE 50 tubing fitted with a silastic tip of 0·30 mm i.d. and 0·64 mm o.d.) was implanted occlusively in the left internal jugular vein. Then either the pulmonary artery proximal to the ductus arteriosus or the ductus arteriosus was ligated on one side of the animal. The side of ligation was chosen randomly. Immediately after surgery, animals were transferred to a Perspex box connected to

a bubble aerator. A pump circulated water between the box and aerator. Water temperature was maintained at 15°C and water P_{O_2} at atmospheric levels. The experimental chamber allowed free access to air. Animals recovered from anaesthesia within 3 h after surgery with no apparent ill effects. One day after surgery, radiolabelled microspheres were infused through the venous catheter. Fifteen minutes after the MS infusion had been completed, the aerator gas was changed from air to 100 % N_2. Water P_{O_2} fell below 10 mmHg (1·33 kPa) within 15 min. One hour after aquatic hypoxia had been initiated, a second set of differently labelled MS was infused into the internal jugular vein. Fifteen minutes later the animal was killed with a 0·3 ml injection of a saturated solution of MS-222. The animal was dissected and the activities of the two MS labels were measured in both lungs and in the rest of the animal. The lung opposite the side of the ligated vessel is referred to as the control lung. MS activity in the two lungs was compared to evaluate the importance of the ligated pathway on lung perfusion during the two different environmental conditions. Twelve neotenes, 10 metamorphosing animals and 15 postmetamorphic animals were used in these experiments.

Controls were performed on seven neotenes. Animals were cannulated as described above, but no vessel was ligated. The following day, two sets of differently labelled MS were infused 75 min apart during normoxic conditions. MS activity was measured in the respiratory sections of the three gill pairs, both lungs and the remainder of the animal. The activities of the two different labels in each tissue were compared to test whether the first injection had affected the MS distribution from the second injection.

Experiments assessing the fraction of cardiac output flowing to the gills and lungs

After the animals had been anaesthetized (MS-222) as described above, cannulae were implanted occlusively in the left internal jugular vein and the left pulmonary vein at the base of the lung. These vessels were too small to permit non-occlusive cannulation. The cannulated lung was removed. After surgery, animals were transferred to a Perspex box maintained at 15°C. The day after surgery, differently radiolabelled MS were simultaneously infused through the two venous catheters. Fifteen minutes after completion of the MS infusions the aerator gas was changed from air to N_2. One hour after the start of aquatic hypoxia, MS with two further labels were infused simultaneously into the two veins and 15 min later the animal was killed. The activities of the four different MS labels was measured in the respiratory sections of the three pairs of gills (neotenes), the remaining lung, the head and the remainder of the animal. The MS infused into the jugular vein are referred to as systemic venous MS, and the MS infused into the pulmonary vein are referred to as pulmonary venous MS. Eight neotenes and eight postmetamorphic animals were used in these experiments.

Time control experiments were performed to determine whether the first set of MS injections affected the distribution of the second set. Seven neotenes were treated as described above, except that water P_{O_2} remained unchanged after the first MS infusions.

Microsphere injection technique

Microspheres (diameter $25 \pm 2{\cdot}5\,\mu m$), radioactively labelled with a gamma-emitting isotope (^{141}cerium, ^{51}chromium, ^{85}strontium or ^{46}scandium) were suspended in a 10 % Dextran/0·05 % Tween-80 solution with an ultrasonic homogenizer. The MS suspensions were transferred to 1 ml syringes and 0·2 ml (containing approximately $2–5\times10^4$ MS) was infused through each cannula over 1 min. MS were kept suspended in syringes during infusion by moving a stainless-steel sphere within the syringe barrel. The cannulae were slowly flushed with 0·1 ml of an amphibian Ringer's solution.

The activities of the different MS labels in each tissue sample were determined by multichannel gamma scintillation counting (Model 5986, Packard Instruments) and on-line microcomputer matrix analysis (see Heisler, Neumann & Maloiy, 1983).

Microspheres with a diameter of $25\,\mu m$ were chosen to allow them to pass through the branchial shunt vessels and pulmonary arterial plexus, but then to become trapped in the tissue capillaries. From vascular corrosion casts of neotenic animals, the maximum diameter of lung capillaries was estimated to be $20\,\mu m$ and the maximum gill capillary diameter to be $15\,\mu m$. The smallest vessels in the branchial shunts and pulmonary arterial plexus were approximately $30\,\mu m$ in diameter (Malvin, 1988). To determine MS entrapment in the branchial shunts and the pulmonary arterial plexus, MS activity in the cartilaginous tissues containing these vascular segments was measured in five neotenes after MS infusion into the jugular vein during both oxygenation conditions. Microsphere recirculation was estimated in five neotenes by injecting MS directly into the systemic arterial system *via* a cannula implanted into the efferent artery of the second gill under the two conditions of aquatic oxygenation, and then counting MS activity in the gills. Under these conditions MS can only have reached the gills by bypassing the systemic microcirculation.

Statistical analysis

The amount of MS in a tissue was expressed as the percentage of total MS infused. In experiments evaluating the two pulmonary perfusion pathways, differences in MS activity between the two lungs were tested by application of a paired *t*-test. For the other experiments, the multivariate Hotelling's T^2-test (Harris, 1985) was used to compare the fraction of pulmonary venous MS in each tissue with that of the systemic venous MS for the initial infusions, the fractions of systemic venous MS infused during the initial infusion period with those introduced during the second infusion period, and the fractions of pulmonary venous MS introduced during the first infusion period with those infused during the second infusion period. For both time control experiments, Hotelling's T^2-test was used to compare the percentages of MS infused initially with the percentages of MS infused 75 min later in each of the tissues examined. Values of $P < 0{\cdot}05$ were considered significant.

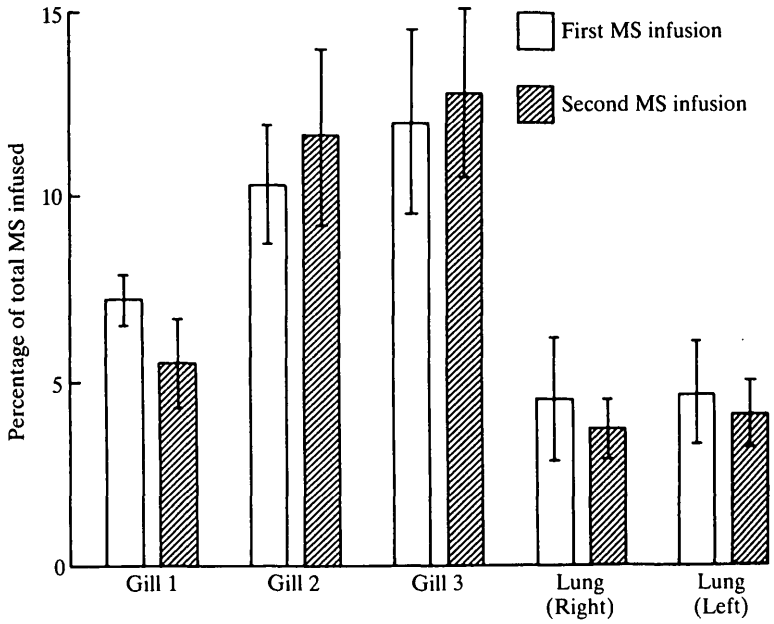

Fig. 3. Distribution of microspheres (MS) in the time control experiments. On the x-axis, 'gill' refers to a pair of branchial respiratory sections ($\bar{x} \pm$ S.E., $N = 7$). There were no significant differences in the fraction of infused MS in each of these tissues between first and second infusion periods ($P > 0{\cdot}14$, Hotelling's T^2-test) or between left and right lungs for either infusion ($P > 0{\cdot}3$ paired t-test).

Results

Experiments assessing microsphere trapping in the branchial shunts and pulmonary arterial plexus, and microsphere recirculation

The cartilaginous tissue containing the branchial shunts of all three gill pairs received $0{\cdot}80 \pm 0{\cdot}18\,\%$ ($\bar{x} \pm$ S.E., $N = 5$) of the MS infused during the initial normoxic period, and $0{\cdot}52 \pm 0{\cdot}07\,\%$ of the MS infused during aquatic hypoxia. The cartilaginous tissues containing both pulmonary arterial plexuses received $0{\cdot}16 \pm 0{\cdot}02\,\%$ and $0{\cdot}29 \pm 0{\cdot}06\,\%$ of infused MS during normoxic and hypoxic conditions, respectively.

During normoxic conditions $2{\cdot}3 \pm 0{\cdot}3\,\%$ ($N = 5$) of the MS infused directly into the systemic arterial system became trapped in the respiratory sections of the gills. During hypoxic conditions $1{\cdot}3 \pm 0{\cdot}1\,\%$ of the MS lodged in the gills.

Experiments to assess the importance of the two pulmonary perfusion pathways

Neotenes

Fig. 3 shows the fractions of infused MS that lodged in the respiratory sections of the three gill pairs and both lungs in the time control experiments. The distribution of the MS infused initially did not differ from the distribution of MS infused 75 min

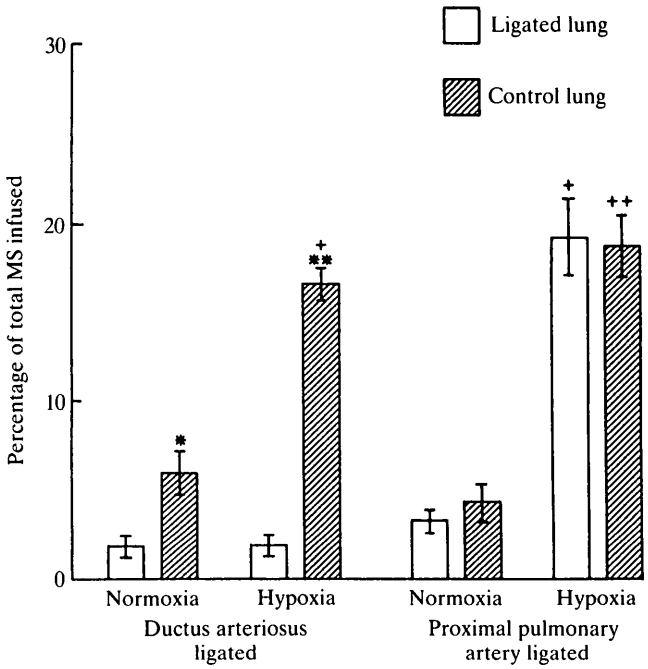

Fig. 4. Fractions of microspheres (MS) lodged in the lungs of neotenic animals ($\bar{x} \pm$ S.E., $N = 6$) during normoxia and aquatic hypoxia with either ductus arteriosus or proximal pulmonary artery ligated, compared with the MS fraction in control (non-ligated) lungs. Asterisks indicate significant differences between control and ligated lungs (*, $P < 0{\cdot}05$; **, $P < 0{\cdot}005$). Plus signs indicate significant differences between normoxia and aquatic hypoxia in the same lungs (+, $P < 0{\cdot}05$; ++, $P < 0{\cdot}005$).

later ($P > 0{\cdot}14$ for all tissues). In addition, the fractions of MS in the left lung did not differ from the fractions of MS in the right lung ($P > 0{\cdot}31$ for either infusion).

In neotenes with one ductus arteriosus ligated, the lung associated with the ligated ductus received 69 % fewer MS than the control lung during normoxia ($P = 0{\cdot}034$). The fraction of MS reaching the control lung during hypoxia was 2·8 times that reaching the same lung during normoxia ($P < 0{\cdot}001$). Aquatic hypoxia did not change significantly the fraction of MS reaching the lung with the ligated ductus arteriosus ($P > 0{\cdot}6$, Fig. 4).

In animals with a ligated proximal pulmonary artery there were no significant differences in the fractions of infused MS between the two lungs during both conditions of aquatic oxygenation ($P > 0{\cdot}25$). Aquatic hypoxia caused the fraction of MS reaching both lungs to increase approximately fivefold ($P < 0{\cdot}013$, Fig. 4).

Metamorphosing animals

Seventy-five percent fewer MS reached the lung with the ligated ductus arteriosus than the control lung during normoxia ($P = 0{\cdot}005$). In animals with the proximal segment of the pulmonary artery ligated, there was no difference in MS

activity between the two lungs ($P = 0{\cdot}12$). Aquatic hypoxia had no effect on the percentage of MS reaching either lung ($P > 0{\cdot}2$, Fig. 5).

Postmetamorphic animals

There was no difference in MS activity between the two lungs when the ductus arteriosus was ligated ($P = 0{\cdot}39$), a pattern not affected by aquatic hypoxia ($P = 0{\cdot}35$). The lungs associated with a ligated pulmonary artery received approximately 40 % fewer MS than the control lungs during both environmental conditions ($P < 0{\cdot}023$, Fig. 6).

Experiments assessing the fraction of cardiac output flowing to the gills and lungs

Neotenes

The time control experiments revealed that injection of the first batch of MS did not affect the distribution of the second set. Significant differences could not be obtained between the distribution of systemic venous MS and pulmonary venous

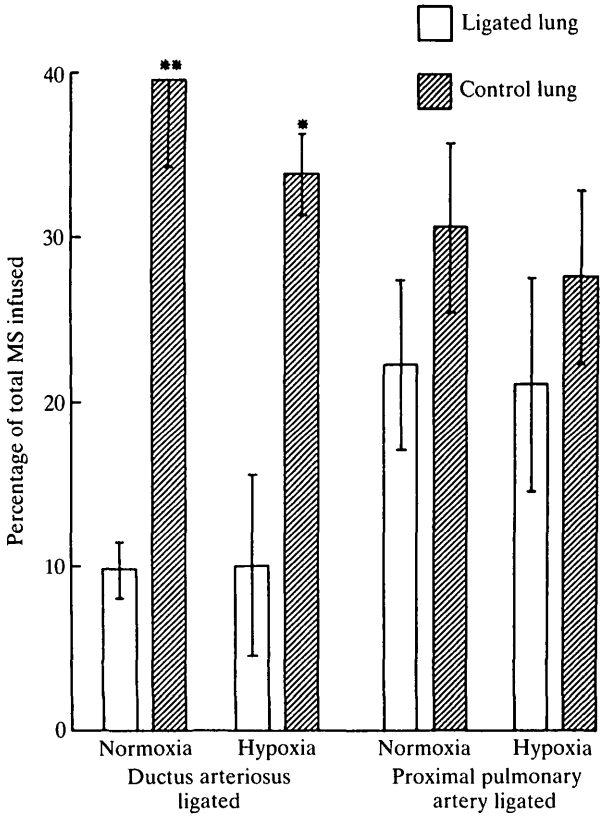

Fig. 5. Fractions of microspheres (MS) in lungs of metamorphosing animals ($\bar{x} \pm$ S.E., $N = 5$). Asterisks indicate significant differences between control and ligated lungs (*, $P < 0{\cdot}05$; **, $P < 0{\cdot}005$). There were no significant differences in MS distribution before and during aquatic hypoxia in the same lungs ($P > 0{\cdot}1$).

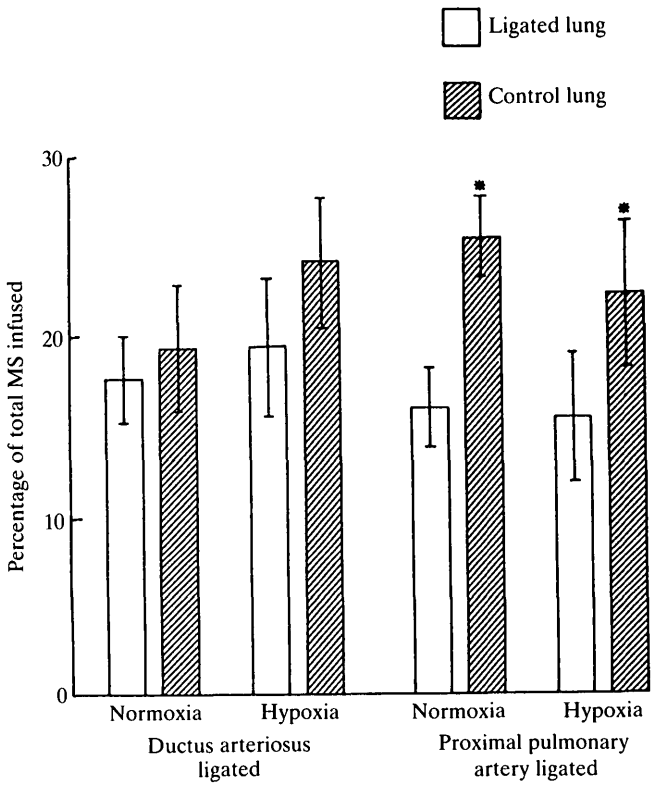

Fig. 6. Fractions of microspheres (MS) in lungs of postmetamorphic animals ($\bar{x} \pm$ S.E.). Asterisks indicate significant differences between control and ligated lungs ($^*P < 0{\cdot}05$). There were no significant differences in the percentages of MS before and during aquatic hypoxia in the same lungs ($P > 0{\cdot}23$). $N = 9$ for animals with the ductus arteriosus ligated; $N = 6$ for the other animals.

MS from the first and the second infusions ($P > 0{\cdot}35$ for each tissue, Figs 7, 8). In addition, the MS activity in each gill was compared with the MS activity in the corresponding gill on the opposite side. For both the first and second injections, there were no significant differences between the right and left sides for the three different gill pairs ($P > 0{\cdot}15$, Hotelling's T^2-test).

To compare systemic venous MS distribution with pulmonary venous MS distribution during normoxia, the data from the first infusions of both the time control experiments and the hypoxia experiments were combined (Fig. 9, $N = 15$). A greater fraction of systemic venous MS than pulmonary venous MS lodged in the respiratory sections of the second ($P = 0{\cdot}017$) and third gill ($P < 0{\cdot}001$) pairs and the lung ($P = 0{\cdot}016$). The respiratory sections of the first gill pair ($P = 0{\cdot}002$) and head ($P < 0{\cdot}001$) received a greater percentage of pulmonary than systemic venous MS, $35{\cdot}5 \pm 3{\cdot}7$ % of systemic venous MS and $25{\cdot}9 \pm 3{\cdot}7$ % of pulmonary venous MS lodged in the respiratory sections of all the gills.

During hypoxia the distribution of systemic venous MS in neotenic animals changed considerably among the different gas exchange sites (Fig. 10). A greater percentage of systemic venous microspheres reached the lung during hypoxia than during normoxia ($P = 0{\cdot}012$). The head ($P = 0{\cdot}036$) and respiratory sections of the first gill pair ($P = 0{\cdot}002$) received a significantly smaller percentage of these MS during hypoxia. The pattern of pulmonary MS was similar (Fig. 11). Differences in the distribution of pulmonary MS between normoxia and aquatic hypoxia were significant in the first ($P = 0{\cdot}044$) and second gill ($P = 0{\cdot}043$) and in the lung ($P = 0{\cdot}010$) and head ($P = 0{\cdot}016$).

Postmetamorphic animals

In postmetamorphic animals, the fraction of systemic venous MS reaching the lung during normoxia was 74 % greater than the fraction of pulmonary venous MS ($P = 0{\cdot}007$). The fraction of pulmonary venous MS that lodged in the head was 70 % greater than the fraction of systemic venous MS ($P = 0{\cdot}05$) during normoxia. The distribution of MS to the head and lung was not affected by aquatic hypoxia ($P > 0{\cdot}14$, Fig. 12).

Discussion

Intracardiac flow of microspheres

Infusion into the jugular vein allowed MS to flow directly into the sinus venosus, then course with systemic venous blood through the heart and into the aortic

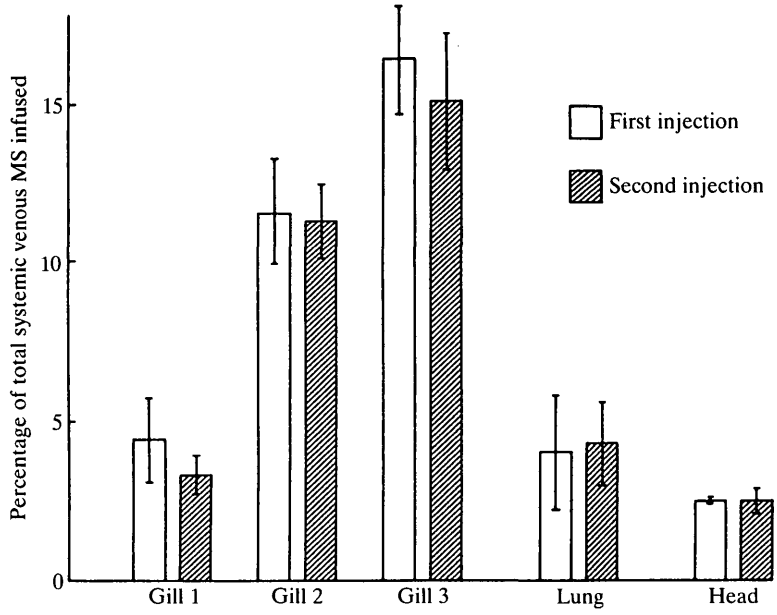

Fig. 7. Distribution of systemic venous microspheres (MS) in the double infusion time control experiments. Values are $\bar{x} \pm$ S.E., $N = 7$. There were no significant differences in the fractions of MS in these tissues between the first and second infusion periods ($P > 0{\cdot}46$).

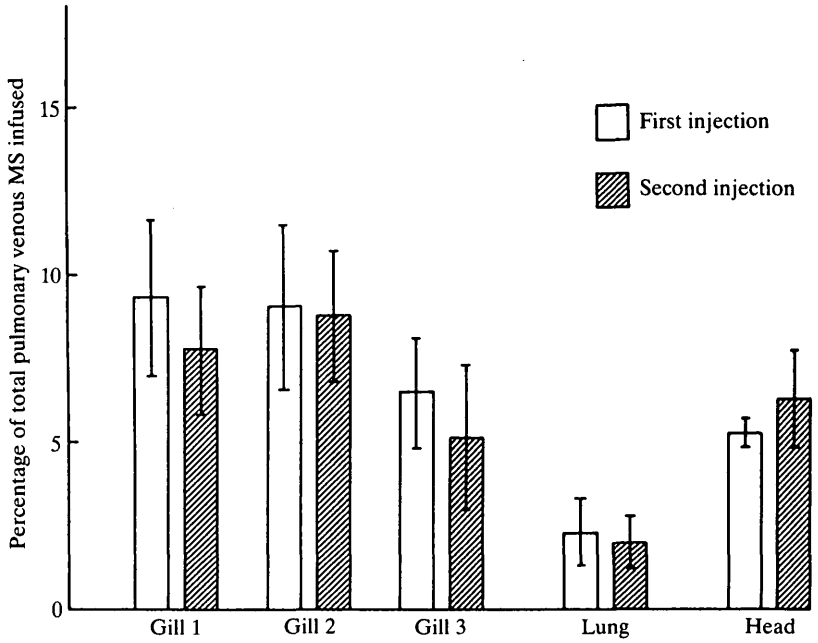

Fig. 8. Distribution of pulmonary venous microsphere (MS) in the double infusion time control experiments. Values are $\bar{x} \pm$ S.E., $N = 7$. There were no significant differences in the fractions of MS in these tissues between the first and second infusion periods ($P > 0{\cdot}35$).

arches. It was assumed that these MS mixed completely with systemic venous blood, and they were consequently considered a marker for the distribution of this blood (i.e. right atrial output). The MS infused into the left pulmonary vein flowed into the common pulmonary vein where they merged with pulmonary venous blood from the right lung. From the common pulmonary vein, MS entered the left atrium. Accordingly these MS traced the distribution of pulmonary venous blood (left atrial output).

Microsphere passage through the branchial shunts and pulmonary arterial plexus, and microsphere recirculation

There was no indication that the MS were too large to pass through the branchial shunts and the pulmonary arterial plexus, since the number of MS trapped in these vessels and surrounding tissues was less than 1 % of the total injected. The experiments in which MS were infused directly into the systemic arterial system indicate that over 90 % of the MS were cleared from the blood during the first pass through the systemic tissues. MS infused into the systemic arterial system flowed with arterial blood and entered the systemic microcirculation. MS which were not cleared by the systemic microcirculation entered the systemic venous system, flowed to the heart and then into the aortic arches. Only

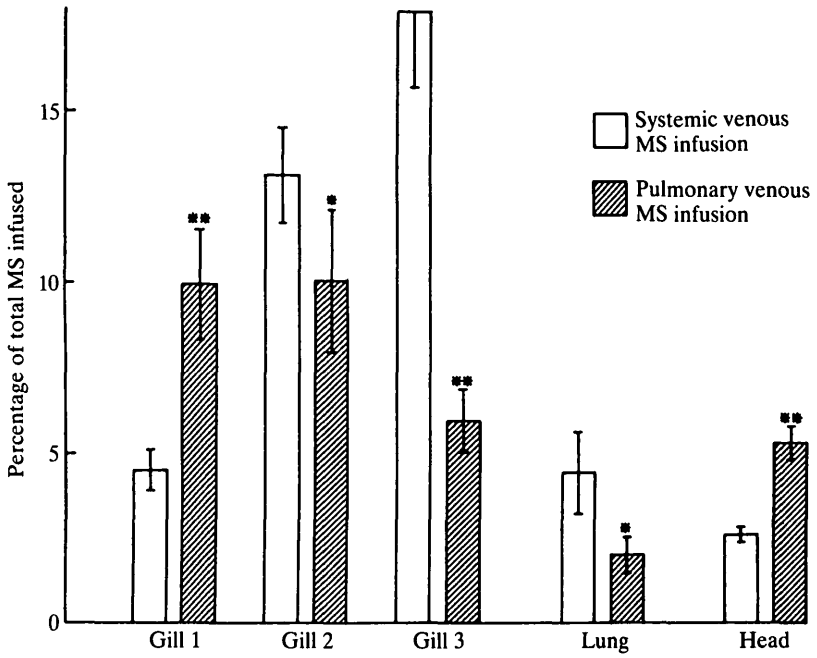

Fig. 9. Distribution of systemic and pulmonary venous microspheres (MS) during normoxia in neotenes. Data from the first infusions of both the time control experiments and the hypoxia experiments are combined ($\bar{x} \pm$ S.E., $N = 15$). Asterisks indicate that the fraction of pulmonary venous MS was significantly different from that of systemic venous MS (*, $P < 0{\cdot}05$; **, $P < 0{\cdot}005$).

2·3 % of these MS lodged in the respiratory sections of the gills under normoxic conditions. In all the experiments, of the MS injected directly into the systemic venous system *via* the jugular vein, 32·6 % lodged in the branchial respiratory sections under normoxic conditions, indicating that this percentage of systemic venous outflow courses through the respiratory sections of the gills. Thus, the fraction of the systemic arterial MS measured in the gills during normoxia indicates that approximately 7·1 % of these MS bypassed the systemic microcirculation. The calculated fraction of MS bypassing the systemic microcirculation during aquatic hypoxia is 5·6 %. It is not known whether these MS passed through systemic capillaries or through arteriovenous anastomoses. In dogs this degree of recirculation does not affect the final distribution of MS to the major organs (Kaihara, Van Heerden, Migata & Wagner, 1968). If recirculation did affect MS distribution in this study, it was assumed that in the pulmonary perfusion pathway experiments recirculation would have an equal effect on both lungs. Consequently, differences in MS activity between the two lungs in an animal were attributed to differences in lung perfusion. In the other experiments, recirculation will have the effect of reducing differences between the distributions of systemic and pulmonary MS.

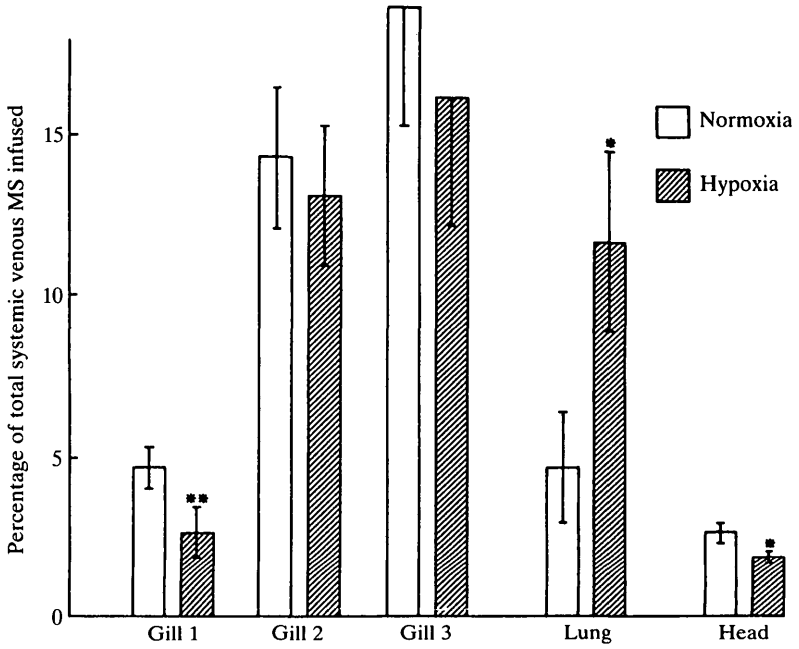

Fig. 10. Effects of aquatic hypoxia on the distribution of systemic venous microspheres (MS) in neotenic animals ($\bar{x} \pm$ S.E., $N = 8$). Asterisks indicate that the fractional distribution of MS during hypoxia was significantly different from that during normoxia (*, $P < 0{\cdot}05$; **, $P < 0{\cdot}005$).

Blood flow patterns during normoxia

Relative importance of the two pulmonary perfusion pathways

It is unknown if ligation of one of the pathways affected blood flow through the remaining pathway. It is, however, unlikely that unilateral vessel ligation would cause flow through the contralateral pathway to decrease. Rather, ligation would probably have either no effect on flow or even augment flow through the patent vessel. An increase in flow through the patent vessel might occur by an active compensatory mechanism, or if vessel ligation caused a decrease in pressure in the distal segment of the pulmonary artery. A decrease in pulmonary arterial pressure would increase the pressure gradient directing flow into the pulmonary artery through the patent pathway. Assuming that vessel ligation did not decrease flow through the patent pathway, a reduction in lung MS activity caused by vessel ligation would indicate that the pathway normally contributes blood to the lung. Inability of vessel ligation to change lung MS activity is difficult to interpret. Such a result would be consistent with an insignificant role of the ligated pathway. However, such a result could also be due to a compensatory increase in flow through the nonligated pathway.

In neotenic animals, ligation of the ductus arteriosus substantially reduced the fraction of MS reaching the lung, whereas ligation of the proximal segment of the pulmonary artery had no effect. These results are consistent with the anatomical

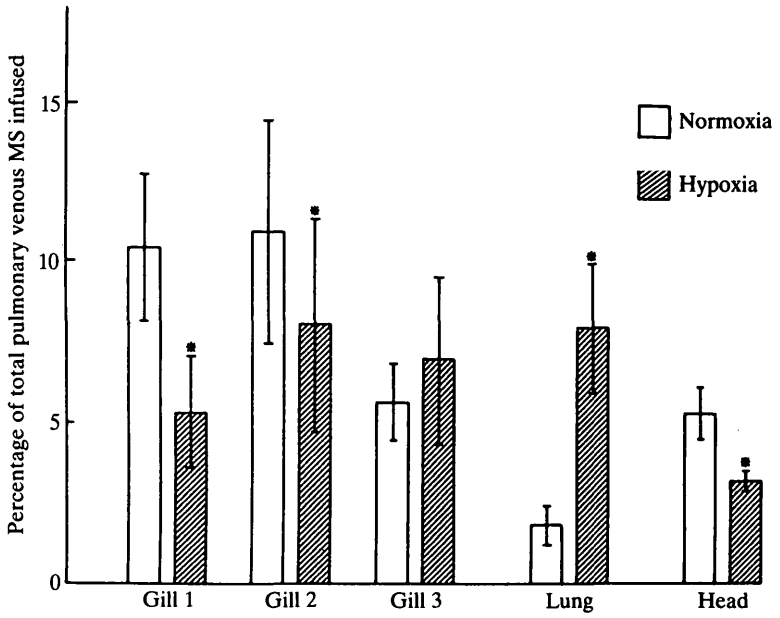

Fig. 11. Effects of aquatic hypoxia on the distribution of pulmonary venous microspheres (MS) in neotenic animals ($\bar{x} \pm$ S.E., $N = 8$). Asterisks indicate that the fractional distribution of MS during aquatic hypoxia was significantly different from that during normoxia (*, $P < 0{\cdot}05$).

evidence indicating that, in the neotene, the major perfusion pathway to the lung is *via* the third gill and ductus arteriosus. The precise fraction of pulmonary blood flow coursing through the ductus arteriosus cannot be determined from this study, primarily because the concentration of MS in the blood flowing through each pathway is unknown. However, flow distribution between the two pathways may be estimated after making several assumptions. (1) Flow through the proximal segment of the pulmonary artery is an insignificant fraction (< 5 %) of total cardiac output. (2) The degree of shunting in each gill is similar. (3) The concentrations of MS in the blood entering the third branchial arch and proximal pulmonary artery are equal. (4) Ligation of the ductus arteriosus has no effect on flow through the proximal pulmonary artery. Assumption 1 is probably correct, since less than 2 % of injected MS reached the lung when the ductus arteriosus was ligated (Fig. 4). Consequently, nearly all MS travelled through the gills. Since approximately 30 % of the total MS became trapped in the respiratory sections of the gills (see Fig. 3), approximately 30 % of total branchial perfusion flows through the respiratory pathway. If assumption 2 is correct, then approximately 30 % of the blood flowing into the ductus arteriosus will have first passed through the respiratory section of the third gill, and consequently will have been cleared of MS. If assumption 3 is valid, the concentration of MS in the blood entering the lung *via* the ductus arteriosus was 70 % of the MS concentration in the blood flowing through the proximal pulmonary artery. If assumption 4 is correct, then

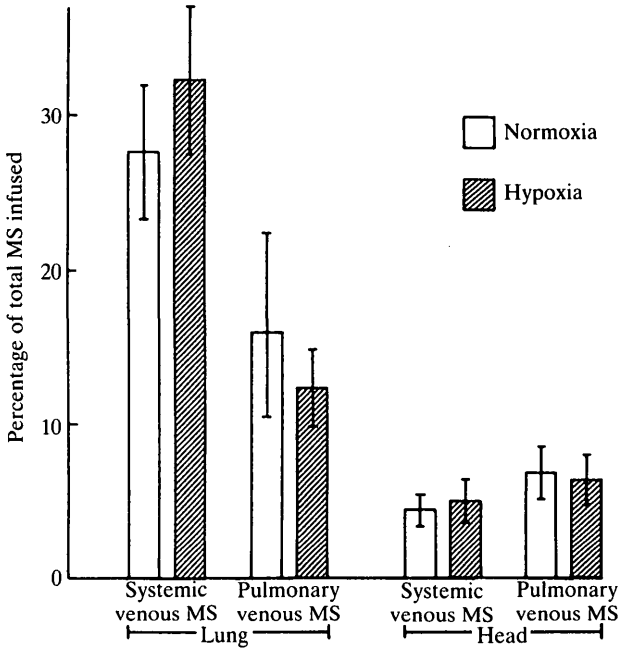

Fig. 12. Distribution of pulmonary and systemic venous microspheres (MS) before and during aquatic hypoxia in the postmetamorphic animals ($\bar{x}$ + S.E., $N = 7$). Aquatic hypoxia had no significant effect on the distribution of MS ($P > 0{\cdot}14$).

the 69 % reduction in lung MS activity after ductus arteriosus ligation (Fig. 4) indicates that approximately 76 % of the blood reaching the lung normally flows through the ductus arteriosus.

It is likely, however, that in the third gill the fraction of blood flowing through the respiratory section is greater than 30 %, because the respiratory section of the third gill is larger, and the shunt vasculature is smaller, than in the other gills. In addition, the concentration of MS in the blood entering the proximal pulmonary artery may have been greater than in the blood entering the third branchial arch, since intracardiac shunting in amphibians usually causes an increase in the amount of systemic venous blood flowing in the anterior compared with the posterior aortic arches. These two considerations suggest that this estimate of fractional flow through the ductus arteriosus is too low. In addition, if ligation of the ductus arteriosus caused a compensatory increase in flow through the proximal pulmonary artery, then normally even more blood flows through the ductus arteriosus. Consequently, it is likely that during normoxic conditions more than 76 % of pulmonary blood reaches the lung *via* the ductus arteriosus.

The experiments in which the proximal pulmonary artery was ligated also indicate that most pulmonary blood flows through the ductus arteriosus. Microsphere distribution was not altered by this ligation, suggesting that very little blood normally reaches the lung directly from the heart. However, it is possible that the lung does receive significant flow from the proximal pulmonary artery, but

compensatory flow through the ductus arteriosus occurs when the proximal pulmonary artery is ligated.

In contrast to the situation in neotenes, ligation of the ductus arteriosus in postmetamorphic animals had no effect on lung MS activity, but ligation of the proximal pulmonary artery significantly reduced lung MS activity. These results also support anatomical evidence that the major pathway to the lung after metamorphosis is from the heart directly down the entire pulmonary artery. Significant flow through the ductus arteriosus may occur, however, since ligation of the proximal pulmonary artery caused only a 40 % decrease in lung MS activity. If the MS concentrations in the blood flowing through the two pathways were similar, then the ductus arteriosus can supply approximately 60 % of the normal pulmonary blood flow when it is the sole pathway to the lung.

Vessel ligation in metamorphosing animals gave results similar to those in neotenes: lung MS activity was reduced by 70 % after ligation of the ductus arteriosus. However, occluding the proximal segment of the pulmonary artery had no effect. This indicates that the shift in lung perfusion patterns had not yet occurred. Since these animals were approximately 75 % of the way through the metamorphic period, pulmonary perfusion patterns must change quite late during metamorphosis.

Distribution of cardiac output to the gills and lungs

In experiments evaluating the distribution of cardiac output, removal of the cannulated lung must have altered the distribution of cardiac output. However, the change in flow distribution was not great enough to cause an unequal trapping of MS between the right and left gill lamellae. This indicates that the experimental procedure did not induce large changes in the distribution of cardiac output into the different branchial arches.

The percentage of systemic venous MS was greater than the percentage of pulmonary venous MS in the respiratory sections of the second and third gill pairs, but a greater fraction of pulmonary venous MS than systemic venous MS was found in the respiratory sections of the first gill (Fig. 9). These differences indicate that the heart of the neotenic tiger salamander, like other amphibian hearts, maintains some degree of separation of pulmonary and systemic venous blood, and then selectively distributes the two pools of blood into the different aortic arches. The precise mixture of pulmonary and systemic venous blood in each arch cannot be determined since absolute flows from the two atria were not measured. However, the MS distributions suggest that blood entering the first branchial arch is rich in pulmonary venous blood but the third branchial arch receives blood enriched with venous blood from the systemic circulation. The greater percentage of pulmonary venous MS than systemic venous MS in the head, and the smaller percentage in the lung, is consistent with the vascular anatomy. The head receives the majority of its blood from the first branchial arch whereas the lung receives most of its blood from the third branchial arch. This arrangement provides the

head with O_2-rich pulmonary venous blood and reduces recirculation of oxygenated blood to the lung.

The MS fractions in the respiratory sections of all the gills indicate that 35·5 % of right atrial output and 25·9 % of left atrial output course through the respiratory lamellar capillaries during normoxic conditions. Since flow down the proximal segment of the pulmonary artery is probably very small, most of the blood bypassing the respiratory sections of the gills probably flows through the branchial shunts. Thus, approximately 25–35 % of branchial blood flow courses through the respiratory lamellae where it is available for gas exchange with the water. In a previous study (Malvin, 1985), measurements of vascular resistance in isolated, perfused gills indicated that approximately 20 % of total branchial flow would course through the respiratory vasculature in the absence of neural or humoral influences. It was further estimated that 8 % of branchial blood flow would perfuse the respiratory lamellae if the respiratory sections were maximally vasoconstricted and the shunts were not constricted. If the branchial respiratory vasculature were maximally dilated and the shunts maximally constricted, it was estimated that 46 % of branchial flow would course through the respiratory sections. If these estimates are accurate, then neural and/or humoral influences are acting *in vivo* to dilate the respiratory sections of the gills and/or constrict the shunts.

The fractions of left and right atrial outputs flowing to the lung are difficult to assess from these data. This is primarily because the fraction of MS reaching the lung is influenced by the unknown fraction of pulmonary blood flow which first passes through the respiratory lamellae of the third gill. However, with the assumptions that blood flow through the proximal segment of the pulmonary artery is negligible and 30 % of the total third gill flow perfuses the respiratory lamellae, 6·3 % of right atrial output and 2·9 % of left atrial output went to the lung. Assuming further that removal of the left lung had no effect on blood flow distribution in the gills and to the right lung, then the lungs normally receive approximately 12·6 % of right atrial output and 5·8 % of left atrial output.

There is no capillary bed between the MS injection sites and the lung in the postmetamorphic salamander. Consequently, MS activity in the lungs of postmetamorphic animals (Fig. 12) is a direct indication of the fractions of the atrial outputs flowing to the lung. Thus, the lung received 27·6 % of right atrial output and 15·9 % of left atrial output under normoxic conditions. Assuming that removal of the left lung had no effect on the fraction of cardiac output flowing to the right lung, then the lungs of intact postmetamorphic animals receive 55·2 % and 31·8 % of right and left atrial outputs, respectively. This is 4–5 times larger than that estimated for the neotene, and is consistent with the greater reliance on pulmonary gas exchange after metamorphosis.

The different percentages of systemic and pulmonary venous MS in the lung and head of postmetamorphic animals (Fig. 12) indicate that, as in the neotene, there is selective distribution of pulmonary and systemic venous blood into the aortic arches, such that recirculation of pulmonary and systemic venous blood to the lung and systemic circulation is reduced. This pattern of flow distribution has been

observed in many other postmetamorphic amphibians (Meyers *et al.* 1979; Johansen, 1963; Johansen & Ditadi, 1966; Tazawa *et al.* 1979).

Blood flow patterns during aquatic hypoxia

In both time control experiments, the MS distributions from the first set of infusions did not differ from the MS distributions from the second set (Figs 3, 7, 8). Consequently, changes in the MS distribution observed under hypoxic conditions must have been the direct result of aquatic hypoxia. It was assumed that the distribution of MS from the second infusion period was not affected by the first infusion in the metamorphosing and postmetamorphic animals.

Aquatic hypoxia causes pulmonary O_2 uptake to increase in neotenic *Ambystoma tigrinum* (Heath, 1976). There are probably many factors contributing to the increase in pulmonary gas exchange. One factor is an increase in pulmonary ventilation. A reduction in water P_{O_2} from about 140–25 mmHg (18·7–3·3 kPa) at 15 °C causes a greater than fivefold increase in pulmonary ventilatory frequency (G. M. Malvin & N. Heisler, unpublished observation). Another contributing factor is probably an increase in pulmonary blood flow. The larger fractions of both systemic and pulmonary venous MS in the lung in both types of experiments during hypoxia suggest that pulmonary blood flow may have been augmented by an increase in the fraction of cardiac output flowing to the lung. However, lung MS activity is not exclusively a function of lung perfusion. As mentioned previously, it also depends on the fraction of pulmonary blood flow that first passes through the respiratory section of the third gill. Since the precise distribution of blood flow between the two branchial perfusion pathways in the third gill is unknown, it is difficult to interpret these lung measurements. However, since the percentage of MS in the respiratory section of the third gill was unchanged by aquatic hypoxia, and since the increase in the fraction of MS activity in the lung was large (between 2·5- and 4·4-fold) it is quite likely that a greater fraction of cardiac output did flow to the lung during aquatic hypoxia, supporting pulmonary O_2 uptake.

In experiments assessing pulmonary perfusion pathways, MS activity in lungs with a ligated ductus arteriosus was 75 % less than in control lungs during normoxic conditions, and 90 % less during aquatic hypoxia (Fig. 4). The larger difference during hypoxic conditions suggests that blood flow through the ductus arteriosus becomes relatively more important during aquatic hypoxia when lung perfusion probably increases.

The fractions of both pulmonary and systemic venous MS in the respiratory section of the first gill pair and the head (which receives most of its blood from the first arch) were substantially less during aquatic hypoxia than during normoxia. In contrast, aquatic hypoxia caused little or no change in the fraction of MS in the respiratory sections of the second and third gills (Figs 10, 11). This suggests that the vascular resistance in the first branchial arch had increased compared with the other arches. Such a change may be adaptive to a hypoxic challenge for two reasons. First, blood will be shunted away from the first gill arches and into the other arches. An increase in flow through the third branchial arch will make more

blood available for lung perfusion *via* the ductus arteriosus. Such a redistribution of blood flow may have contributed to the increase in the percentage of MS reaching the lung during aquatic hypoxia. Second, a decrease in blood flow through the first gill should reduce O_2 loss from blood to water across the respiratory surface of that gill during severe aquatic hypoxia. This will be more important in the first gill than in the other gills since blood entering the first branchial arch should contain the highest proportion of oxygenated pulmonary venous blood.

Since aquatic hypoxia increases pulmonary O_2 uptake in postmetamorphic animals (Heath, 1976), lung perfusion in these animals was expected to increase during hypoxia. However, the percentage of MS reaching the lungs in both types of experiments was not affected by aquatic hypoxia in metamorphosing and postmetamorphic animals (Figs 5, 6, 12). It is possible that lung perfusion did increase *via* a rise in cardiac output without a concomitant change in the distribution of cardiac output. This, however, is highly speculative since absolute blood flow cannot be determined from this study. The reason for the difference between neotenes and the metamorphosing and postmetamorphic animals in this regard is unknown.

In conclusion, the ductus arteriosus is the major perfusion pathway to the lung in neotenes and metamorphosing animals that have experienced approximately a 75 % reduction in gill mass. After metamorphosis is complete, the major pathway to the lungs appears to be the direct route from the heart down the entire length of the pulmonary artery. The ductus arteriosus, however, is still able to supply a significant amount of blood to the lungs if the proximal pulmonary artery is ligated, suggesting that it may still serve as an important respiratory vessel. The shift in lung perfusion from the ductus arteriosus to the pulmonary artery occurs very late in metamorphosis. During severe aquatic hypoxia, blood flow through the ductus arteriosus probably becomes even more important in neotenes. There is no evidence that aquatic hypoxia alters the pattern of blood flow to the lungs in metamorphosing and postmetamorphic animals.

The posterior aortic arches and the lungs receive blood richer in systemic venous blood than the anterior arches. Approximately 26 % of the left atrial output and 36 % of the right atrial output perfuse the respiratory lamellae of the gills under normoxic conditions in neotenes. In these animals, severe aquatic hypoxia appears to elicit an increase in the fraction of cardiac output flowing to the lungs and a decrease to the first branchial arches. A decrease in fractional flow into the first arch should make more blood available for lung perfusion. In addition, it should reduce the flow of O_2-rich blood through the respiratory lamellae of the first gill, limiting branchial O_2 loss. 55 % of right atrial output and 32 % of left atrial output flow to the lungs in the postmetamorphic animals. Aquatic hypoxia does not elicit a redistribution of cardiac output in the postmetamorphic animals.

Supported by Deutsche Forschungsgemeinschaft.

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