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# FACTORS AFFECTING BLOOD FLOW TO THE LUNGS IN THE AMPHIBIAN, *XENOPUS LAEVIS*

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## INTRODUCTION

Studies on the physiology of the pulmonary and systemic circulations in amphibia, particularly in relation to gas exchange, have so far been concentrated largely on determination of blood pressures (Shelton & Jones, 1965, 1968), heart rate (Jones & Shelton, 1964; Jones, 1968), and partial pressure of respiratory gases in the two circuits (Johansen, 1963; Johansen & Ditadi, 1966; Toews, Shelton & Randall, 1971). It is clear that all these parameters change considerably if an amphibian stops breathing air and relies entirely on cutaneous gas exchange as it does, for example, during a dive. Bradycardia has been described in a number of amphibians during periods of submersion and has been considered as part of a more general phenomenon in which the basal metabolic rate is lowered as oxygen becomes less available. Part of the same overall effect is a redistribution of blood flow which is known to occur in some species, with an increase of cutaneous flow (Poczopko, 1960) probably increasing gas exchange through the skin. The fall in heart rate and heart output is not, however, accompanied by equivalent falls in arterial blood pressure so that the overall vascular resistance of the periphery must increase in spite of cutaneous vasodilation (Shelton & Jones, 1965). Though these alterations appear to be linked in some way to the relative oxygen lack, little is known about the way in which the responses are mediated (Jones, 1967).

In the course of recent studies on the respiratory function of amphibian circulation more specific evidence than had hitherto been available emerged to show that one part of the peripheral circulation, namely that of the lungs, was affected in a very precise and reproducible way by periodic fluctuations in certain aspects of the overall gas exchange (Shelton, 1970). It was found that a breathing period in the toad, *Xenopus laevis*, was accompanied by a marked increase in pulmonary blood flow, simultaneously with a decrease in pulmocutaneous arterial pressure. These changes seemed dependent on lung ventilation since they were not observed if the breathing movements were ineffective in actually ventilating the lungs. They imply a considerable vasodilation of the pulmocutaneous vascular bed, presumably affecting the lung vessels rather than the skin. The experiments were carried out and the changes were observed when the animal was breathing normally in air; it was suggested that fluctuations of a similar nature but of extended duration occurred when the animal was alternately surfacing and diving.

Several hypotheses can be put forward to explain the pulmonary vasodilation response. It may be simply dependent on the mechanical deformation of the lungs, or it

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may be due to a more complex system which could be entirely local or have some central components. The effective trigger to either a local or a reflex system could be lung inflation itself. Alternatively, changes in gas tensions in the lungs or in the blood, detected by appropriate chemical receptors, may be more important.

The following experiments were designed to test some of these possible mechanisms. Measurements of systemic and pulmocutaneous blood pressure and flow were taken under different respiratory conditions, obtained by artificially filling the lungs with gases of different oxygen content. The effect of blocking the peripheral vagus innervation was also observed.

## METHODS

The experiments were carried out, at room temperature (20 °C), on twenty-three healthy female *Xenopus laevis* weighing between 90 and 115 g. The animals were anaesthetized by immersion in an aqueous solution of Tricaine (400 mg/l) for 45–60 min followed by a subcutaneous injection of Nembutal (3 mg). It was found in preliminary experiments that it was difficult to maintain a steady state of light anaesthesia if Tricaine alone was used. The animals became restless and did not tolerate some aspects of the artificial ventilation procedure, particularly if their mouths were kept open as required in some of the experiments. Under these conditions they made frequent attempts to breathe, and evaluation of the results became very difficult. The small dose of Nembutal depressed the respiratory centre to an extent which permitted the observation of effects of artificial inflation of the lungs without a large number of superimposed breathing movements.

The surgical approach and instrumentation used to measure the arterial blood flows and pressures has been described elsewhere (Shelton, 1970). Briefly, blood pressures were measured by means of Sanborn 267B manometers connected directly to the arterial arches by 25 cm lengths of flexible nylon tubing of 0·5 mm bore. These cannulae were inserted into the lumen of the vessels with their openings directed downstream. The internal diameter of the arteries was some 2–3 times greater than that of the cannulae so that there was partial but never complete obstruction of flow. A Biotronix BL 410 electromagnetic blood flowmeter was used to measure blood flow. The flow probes were selected to be a good fit on the vessels concerned and had apertures ranging from 1·0 to 2·0 mm. The probes were located on the pulmocutaneous arch of one side and the systemic arch of the contralateral side, as it was not possible, because of their size, to have both probes on one side. An inverse arrangement was used for the pressure cannulae. Control experiments established that all the effects recorded were similar on both sides of the animal so that no complication was introduced by this method of recording.

Before exposing the arterial arches a small incision was made on the right flank of the animal, and the posterior region of the right lung was cannulated with a short length of flexible tubing of 1 mm bore. This could be connected to a syringe so that gas could be introduced into the lungs and samples of lung gas could be taken and analysed for oxygen content. A Radiometer E 5046 electrode connected to a Beckman Gas Analyser Model 160 was utilized for $P_{O_2}$ determinations. The sample size was 0·3–0·5 ml.

A volume of 5 ml of air or nitrogen was generally used to fill the lungs, though

smaller amounts were occasionally required, as specified in the following section. The mouths of the toads were kept open a little during the experiments to prevent the normal filling of the lungs, but breathing movements were not mechanically restricted. The glottis was normally closed firmly, though it was left free to open. If the lungs were inflated with volumes greater than 7–8 ml, it was found that the glottis would open and release some of the gas. Some of the preliminary lung-inflation experiments were effected via a cannula in the glottis; the results from these were identical with those from the posterior lung cannulations.

In order to confirm that the changes described in these acute preparations were also found in more normal conditions, another group of experiments was carried out to observe the blood-pressure changes when the animals were swimming freely, without the influence of anaesthetics. Instead of cannulating the main arterial arches, branches of the cutaneous and femoral arteries were exposed, and the cannulae were tied safely in place. The cutaneous artery was approached as it emerged between the skull and the scapula and ran to the skin. The surgical incisions were closed and the animals were allowed to recover from the anaesthesia. The blood pressures were then recorded by the usual methods while the unrestrained animals moved about a small tank. Flow measurements were not possible under these conditions because of the large volume of the probes, which could not be chronically implanted.

## RESULTS

When the lungs were filled with 5 ml of air, the changes in pulmocutaneous blood pressure and flow were identical to the changes following normal breathing, though their amplitudes were exaggerated. A sharp decrease of the blood pressure could be seen, lowering both systolic and diastolic pressures by as much as 15–20 cm $H_2O$ (Fig. 1). This was sometimes followed by a further slow drop of the diastolic pressure, so that the pulse pressure gradually increased. The pulmocutaneous blood flow rose sharply when the lungs were inflated, and often continued to increase slowly until a maximum was reached after 2–4 min. Peak flow rates could be six to ten times greater than before inflation, rising from 20–30 $\mu$l/sec to 180–200 $\mu$l/sec. This situation was maintained for as long as 10–20 min, during which only a slight decrease of pulmocutaneous blood flow and increase in pulmocutaneous blood pressure were seen. By that time the partial pressure of oxygen in the lung gas would have fallen to between 50 and 70 mmHg. The toads then generally breathed out and the initial pressures and flows were rapidly restored.

Systemic blood flow remained unchanged except for a variable, small and transient decrease when the lungs were inflated. The systemic pressure showed an initial decrease following lung inflation, but the change was smaller than for pulmocutaneous pressure and no increase in pulse was observed. Systemic pressures were on the whole higher than those recorded in the pulmocutaneous arch (Figs. 1–4). A difference in diastolic levels in the two arches is to be expected since the valves at the top of the conus are closed at this stage and diastolic run off will be determined independently in the two systems by the respective values for compliance and peripheral resistance. The difference in systolic pressures cannot be so easily interpreted since the recording sites are both very close to the common, ventricular pressure source. Indeed, previous

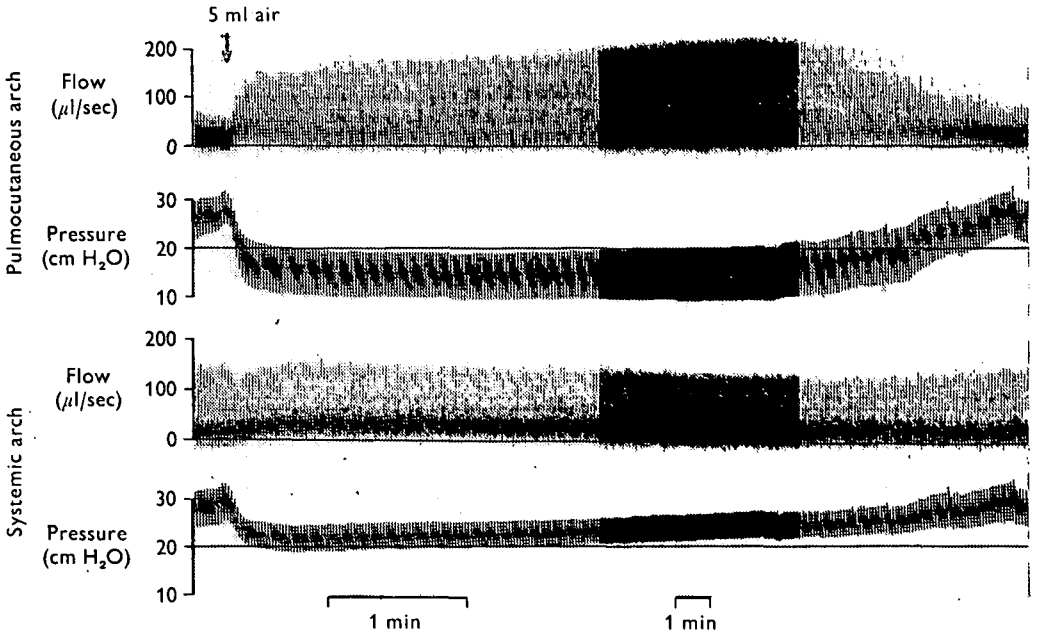


Fig. 1. Effect of lung inflation with 5 ml of air on the blood pressures and flows in the pulmocutaneous and systemic arches of *Xenopus*. The arrow indicates time of inflation.

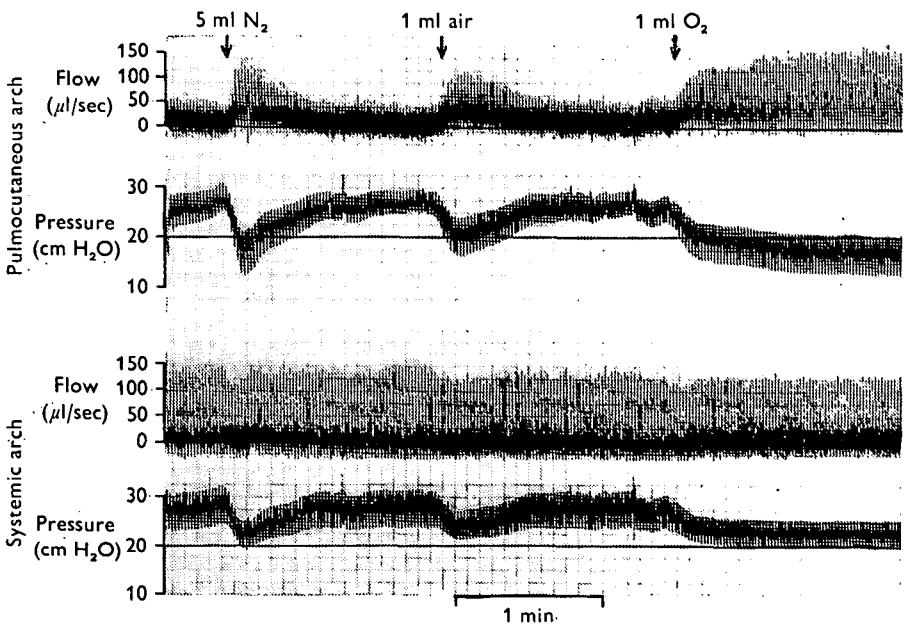


Fig. 2. Effect of lung inflation with different gases on the blood pressures and flows in the pulmocutaneous and systemic arches of *Xenopus*. The arrows indicate time of inflation with 5 ml of nitrogen, 1 ml of air and 1 ml of oxygen.

work has established that no difference in systolic pressures is found if the recording techniques are adequate (Shelton & Jones, 1968). The lower pulmocutaneous pressures recorded in the present experiments must be due to the obstruction and pressure drop consequent on the introduction of a cannula into the vessel. This effect is not so marked in the systemic arch because an unobstructed channel to the cannula exists via the

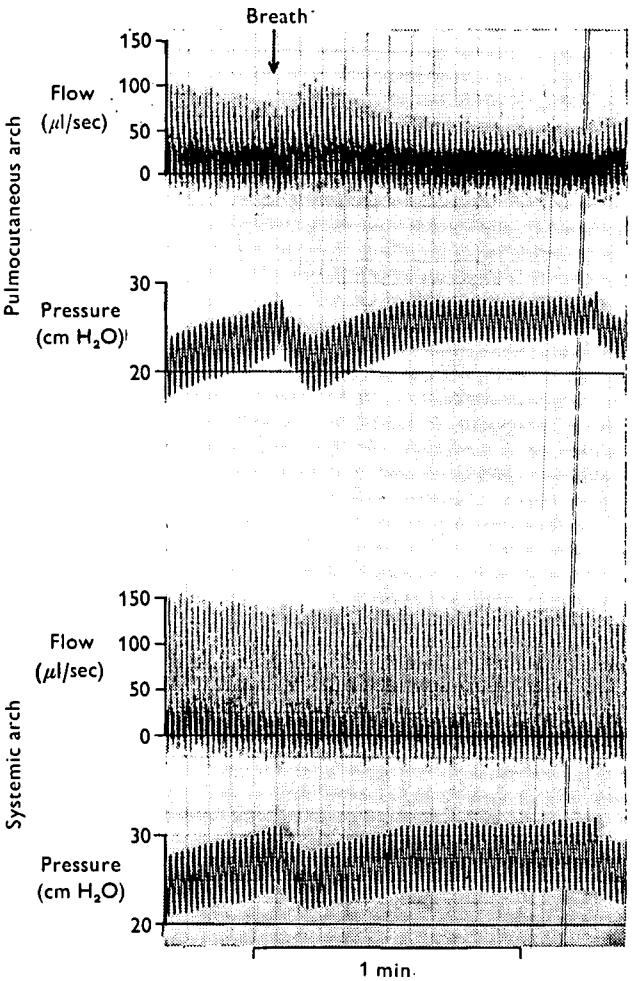


Fig. 3. Effect of a buccal breathing movement that was ineffective in ventilating the lungs on blood pressures and flows in the pulmocutaneous and systemic arches of *Xenopus*. The arrow shows the time when the animal tried to breathe.

dorsal aorta connexion with the contralateral systemic arch. If this hypothesis is correct then it might be expected that the differences would become more marked as the blood pressures fell and the pulmocutaneous obstruction assumed progressively greater proportions. Figs. 1–4 show that this is indeed the case. Finally, Fig. 6 offers striking evidence that the systolic pressures in both systemic and pulmocutaneous systems are in fact identical at recording sites much closer to the periphery than those chosen for the other records.

In contrast with the long-lasting effects of introducing air into the lungs, inflation with the same volume of nitrogen was followed by transient and somewhat smaller changes in pulmonary pressures and flow (Fig. 2). The drop in pressure could be of the same order as seen with air for the first few heart beats after inflation, but this trend was almost immediately reversed towards the initial values. The changes in blood flow showed a similar pattern, again returning to the initial values in about one minute. No attempt was made to remove the residual air prior to introducing nitrogen in the lungs, and the partial pressure of oxygen obtained in these conditions was between 20 and 35 mmHg.

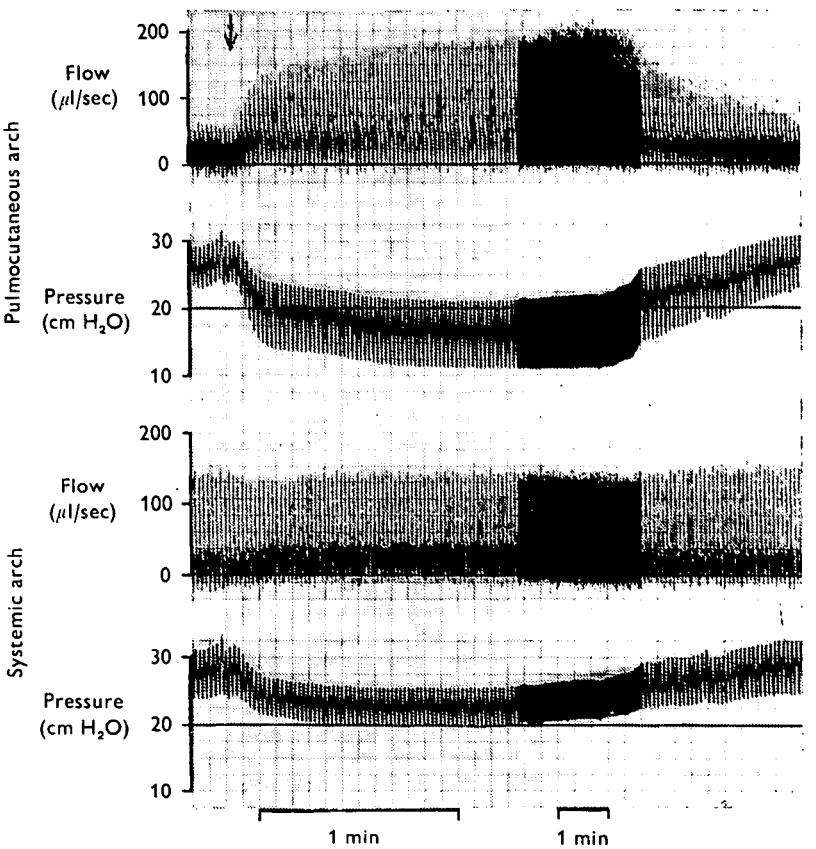


Fig. 4. Effect of lung inflation with 1 ml of oxygen on the blood pressures and flows in the pulmocutaneous and systemic arches of *Xenopus*. The arrow shows the time of inflation.

The pattern of responses was modified by the level of anaesthesia. If the animal was anaesthetized to the point of being unable to make spontaneous breathing movements, inflation of the lungs with air had but a small effect on the pulmocutaneous blood flow. On the other hand, if the toad was only lightly sedated, its attempts to breathe when the mouth was kept slightly open were followed by a marked increase of the pulmocutaneous blood flow and decrease of the pressure. These changes could be maintained for a relatively long period by repeated movements of the buccal cavity even though such movements were totally ineffective in lung ventilation. Most of our

experiments were performed with the animals in a state intermediate between these two so that the effect of artificial filling of the lungs could be observed without the superimposed effect of breathing movements, but the animals were still able to breathe spontaneously, and would do so when the partial pressure of oxygen in the lung fell below 50–80 mmHg. In this situation a single open-mouth breathing movement had a small transitory repercussion in pulmocutaneous blood flow and pressure, similar to the effect of nitrogen (Fig. 3).

Inflation of the lungs with comparatively small volumes of air (1–2 ml) induced smaller and shorter lived changes in pulmocutaneous blood pressure and flow while the use of the same volume of oxygen produced an effect approximating to that of full inflation with 5 ml of air. This is shown in Fig. 2, where the effects of lung inflation with nitrogen, 1 ml of air and 1 ml of oxygen can be compared.

The partial pressure of oxygen in the lung gases after filling with 5 ml of air was usually between 120 and 140 mmHg, while it reached much higher values (250–380 mmHg) when 1 ml of oxygen was used. Nevertheless the peak values for pulmocutaneous blood flow tended to be slightly smaller in the latter case and were reversed within a shorter time. For example, in the experiment illustrated in Figs. 2 and 4 lung inflation with 5 ml of air would be followed by a rise in pulmocutaneous blood flow with peak values around 250 $\mu$l/sec and sustained for 10–12 min, while inflation with 1 ml oxygen would induce peak flow values of 200 $\mu$l/sec for 6–8 min.

The variation of heart rate that is normally observed in *Xenopus* in connexion with the breathing behaviour (i.e. a gradual decrease in heart rate during the periods of apnoea followed by a sudden recovery when breathing occurs) was not elicited by periodic artificial inflation of the lungs. Heart rates varied between 40 and 55 beats/min in the different animals but remained without appreciable alterations in each case throughout the various inflation procedures.

In eight of the animals atropine (0·1 mg) was injected subcutaneously in order to study the effect of vagus blockage on the responses of the pulmocutaneous circulatory system. A few minutes after the injection a rise in blood flow was observed in the pulmocutaneous arch accompanied in some cases by an increase in pulse pressure. Pulmocutaneous blood flow was then maintained at very high, probably maximal, levels even after long spells of apnoea. The systemic flow was not altered in a consistent manner by the atropine injections, and any changes that did occur were much smaller than those found in the pulmocutaneous vessel. Usually, no changes in pulmocutaneous flow were induced by normal breathing after atropine injection. Under these conditions, however, breathing always had a measurable effect on the blood pressures, which fell by 1–2 cm $H_2O$ in both circuits. Occasionally an equivalent small drop in the flow pulses could be seen, but the effect was so small as to be at the limit of resolution of the flowmeter. The effect was much more obvious when the lungs were artificially inflated, as can be seen in Fig. 5. Here an injection of 5 ml of air into the lungs reduces both the systolic pressure and pulse size in the pulmocutaneous artery whilst the diastolic pressure is not much altered. There appears to be a decrease in blood flow equivalent to the fall in mean blood pressure suggesting little or no peripheral change.

Blood-pressure changes observed in animals with chronically implanted cannulae after they had recovered from anaesthesia were essentially similar to the changes previously described (Fig. 6). A sharp decrease of the cutaneous artery pressure was

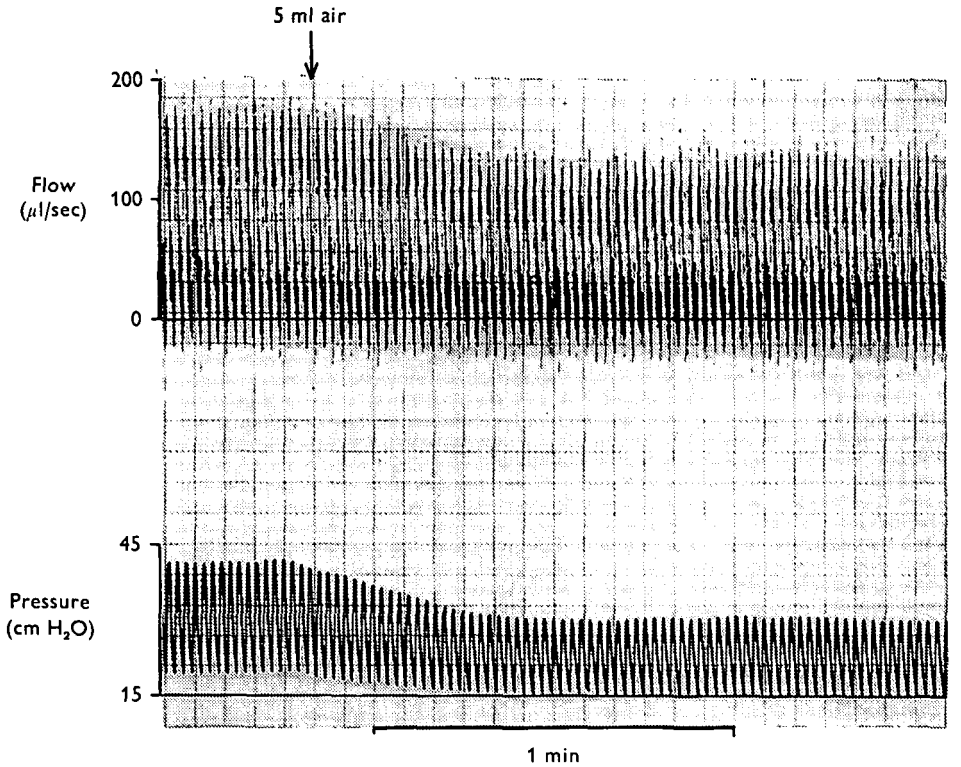


Fig. 5. Effect of lung inflation with 5 ml of air on the blood pressure and flow in the pulmocutaneous arch of an atropinized *Xenopus*. The arrow shows the time of inflation.

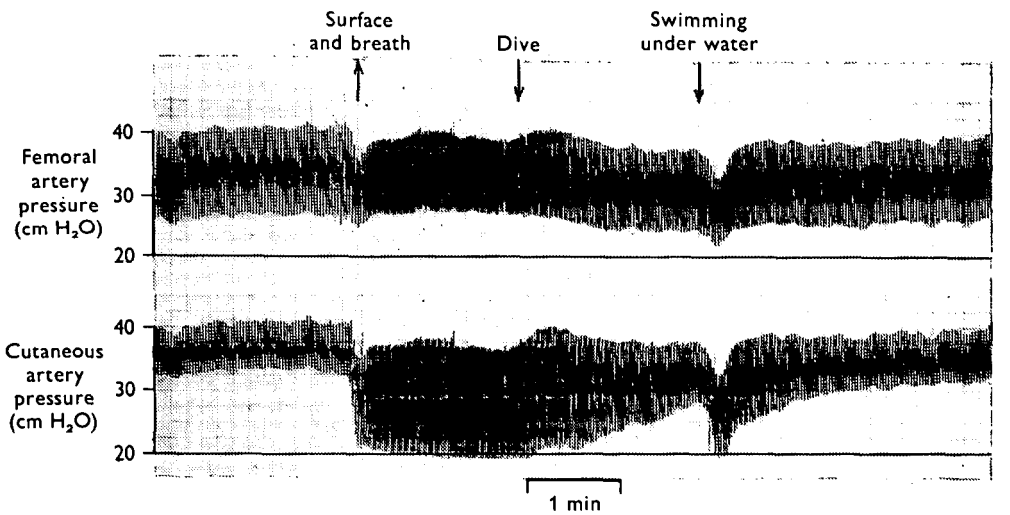


Fig. 6. Blood pressures in a femoral and a cutaneous artery of a free-swimming *Xenopus*, showing the effects of surfacing, diving and swimming.

recorded when the animal emerged to breathe. The diastolic values were the most affected, with a corresponding increase of the pulse pressure. The changes in the body system were much less marked and were transitory, while the increase in the pulmocutaneous pulse pressure lasted throughout the breathing period and was reversed slowly after submergence. Transient decreases of both systemic and pulmocutaneous blood pressures were observed when the animals moved under water, and a slight undulation of the mean pressure values was seen even during resting periods. These phenomena are also illustrated in Fig. 6, which reproduces a record taken in one of these experiments. It is clear from these experiments that the changes in pressure and flow described for the acute preparations must also occur in the intact animal during the course of normal diving–emergence behaviour.

## DISCUSSION

The results of the experiments described above confirm the former observations (Shelton, 1970) on the changes induced in the pulmocutaneous circulation of *Xenopus* by lung ventilation, and suggest that the mechanisms involved are quite complex. The effects of artificial inflation of the lungs were essentially similar to those following normal breathing movements, involving a substantial vasodilation of the pulmonary vascular bed. The fact that these changes could be induced by passive filling of the lungs seemed to point to the existence of a mechanical factor as the basis of the phenomenon. This has been alleged to explain a drop in vascular resistance produced by the expansion of the lungs in mammals, the most probable site of change being the pulmonary capillaries (Bolton *et al.* 1969).

On the other hand, the diminished response obtained when the same volume of an inert gas was used instead of air indicates that the presence of oxygen is essential for the full development of the vasodilation reaction. However, oxygen *per se* does not produce as big an effect as a larger volume of air with the same oxygen content, though the partial pressure of the gas in the lungs may be very large indeed in the former case. From the assembly of these facts it seems to emerge that both the mechanical deformation of the lungs and the oxygen content of lung gases are important factors for the development of pulmonary vasodilation. The possibility that accumulation of carbon dioxide might account for the variation of pulmonary blood flow is excluded by the small effect of nitrogen, which would be at least as effective as air in diluting or removing the gas; besides, it is reasonably well established that most of the metabolic carbon dioxide is removed through the amphibian skin and does not accumulate appreciably in the lungs (Foxon, 1964).

Another interesting feature of our results is the fact that the level of anaesthesia plays an important role in determining the degree of response to the different stimuli. For example, in a lightly sedated animal, normal breathing movements made ineffective by keeping the mouth open could induce a considerable increase in pulmonary blood flow, while at a deeper level of anaesthesia even full lung inflation with air had a very small effect. The most plausible explanation for these facts is that the nervous system has a predominant role in the development of circulatory changes induced by breathing activity, which will be presumably mediated by central reflex mechanisms.

Though the simple mechanical deformation of the lungs may by itself alter the

vascular resistance and perhaps explains the small increase in blood flow when the lungs of a deeply anaesthetized animal are inflated, the full vasodilation reaction is only seen in the animals whose reflexes are not completely abolished or, better still, in animals which are not under anaesthesia. An earlier hint of the existence of a central regulation of the pulmonary blood flow of frogs was provided by Bastert (1929), who produced evidence to show that there is some form of control of the number of lung capillaries open at any moment, which is lost when the animal is pithed.

Little is known about the actual nervous paths involved in the process. The departing points for the vasodilation reaction must be either the stretch receptors in the lungs or chemical receptors sensitive to the partial pressure of oxygen in the lungs or in the blood, or both. The relative importance of these two factors cannot be sorted out from our results, though the intensity and duration of the response to pure oxygen suggest that the chemical factor is the fundamental one. This is in good agreement with results described in other animals. Ventilation hypoxia elicits increased pulmonary vascular resistance in several mammalian species (Hauge, 1968); the alveolar $P_{O_2}$ seems to be the main determinant for the vasoconstriction effect in rats, though pulmonary arterial hypoxaemia is also important (Hauge, 1969).

It appears from the effect of vagus blockage that the vagus system is also involved and that the gradual regression of the pulmonary vasodilation is a product of vagal activity. Assuming that variable blood flow to the lungs is typical of the intact animal and related to diving periods, as suggested by our results after recovery from anaesthesia, this could be understood as part of a generalized tendency towards lowering the metabolic rate and economizing the oxygen contained in the lungs during a dive. Preliminary determinations of the pulmonary oxygen content after lung inflation with air showed that the rate of lung-oxygen consumption was at its highest after inflation and immediately started to decrease. It would be interesting to relate this fact to total oxygen consumption and pulmonary blood flow during submergence. Jones (1967) found that several species of anurans, including *Xenopus laevis*, consumed less oxygen during diving periods than on emergence, and showed that this depended on the amount of oxygen available in the water. From his results it is evident that regulation of cutaneous oxygen exchange is a complex function, and this fact must be taken into consideration in further studies on lung ventilation–perfusion relations in amphibia.

The results of our experiments may be compared to what is known to occur in higher animals submitted to conditions of hypoxia. In diving mammals submersion evoked an oxygen-conserving reflex pattern which included bradycardia, decreased myocardial contractility and peripheral vasoconstriction (Andersen, 1966). It was found that atropinization prevented the full development of this phenomenon, suggesting that vagal activity was critical to oxygen conservation (Ferrante, 1970). Presumably the pulmonary vasoconstriction during apnoea periods in *Xenopus* has a parallel meaning, and, as we saw, it was also prevented by atropinization.

## SUMMARY

1. A series of breathing movements which are effective in ventilating the lungs are accompanied by a marked increase in pulmonary blood flow and a decrease in pulmocutaneous arterial pressure. This reaction must involve considerable vasodilation of the pulmonary vascular bed.

2. Similar vasodilation is produced by artificial inflation of the lungs via an implanted cannula. Nitrogen is not so effective as air in producing the vasodilation, whereas oxygen is more effective. It is suggested that both stretch receptors in the lungs and chemical receptors in lungs or blood are involved in the reaction.

3. The level of anaesthesia is important in determining the degree of vasodilation response to the different stimuli. It is concluded that the central nervous system is the site where interaction occurs between signals from stretch receptors and chemical receptors and from the breathing movements themselves. Experiments with atropine suggest that the efferent pathway is in the vagus nerve.

4. Recordings from free-swimming, unanaesthetized toads show that the vasodilation response occurs as part of normal diving-emergence behaviour.

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