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GAS EXCHANGES AND BLOOD GAS CONCENTRATIONS IN THE FROG *RANA RIDIBUNDA*

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INTRODUCTION

It is well known that Amphibia have two main respiratory surfaces, the lung and the skin. However, not much information is available on the relative importance of these two surfaces for the gaseous exchanges or on the modification of the respiratory pattern during diving periods. This problem has to be tackled separately in different species, as the anatomical characteristics of the respiratory and circulatory systems and the living habits vary considerably in different forms. A review of earlier publications dealing with these aspects may be found in Foxon (1964).

It has been generally accepted that the lungs are the main pathway for the absorption of oxygen in anura, whereas the main respiratory function of the skin would be the elimination of carbon dioxide. This being so, one might expect that prolonged periods of immersion would give rise to a progressive decrease of partial pressure of both gases in the blood, since there would be a large fall in oxygen uptake whereas the capability for carbon dioxide elimination ought to be substantially unchanged. This hypothesis would be wrong if there were some unpredicted variations in the properties of the gas exchangers, particularly the skin, or in the relationship between oxygen uptake and carbon dioxide production of the metabolizing cells. Certainly, an important modification in the lung circulation during diving has been described in *Rana catesbiana* (Johansen, Lenfant & Hansen, 1970) and in *Xenopus laevis* (Shelton, 1970; Emílio & Shelton, 1972). It was found that the vascular impedance of the pulmocutaneous territory is very high during immersion, leading to a dramatic decrease of the blood flow down to one tenth of the emergence levels. It was assumed that only the lung epithelium was affected by this phenomenon, but no direct evidence of any change in skin circulation could be obtained. Experiments by Poczopko (1959) in *Rana esculenta* actually showed an increase in the number of open skin capillaries during diving.

The following experiments were designed to assess the cutaneous and pulmonary gas-exchange capacity in frogs and to study the changes in the blood gas concentrations during prolonged diving periods.

METHODS

Frogs of the species *Rana ridibunda* (Pallas) weighing between 35 and 60 g were used. They were kept without food in a cold room (8 °C) for 5–8 days, and acclimated to room temperature (22–24 °C) for 2 h before starting the experiments. Their cloacas were ligated beforehand to avoid contamination of the external medium with urine or faeces. The determinations of oxygen intake and carbon dioxide output were carried

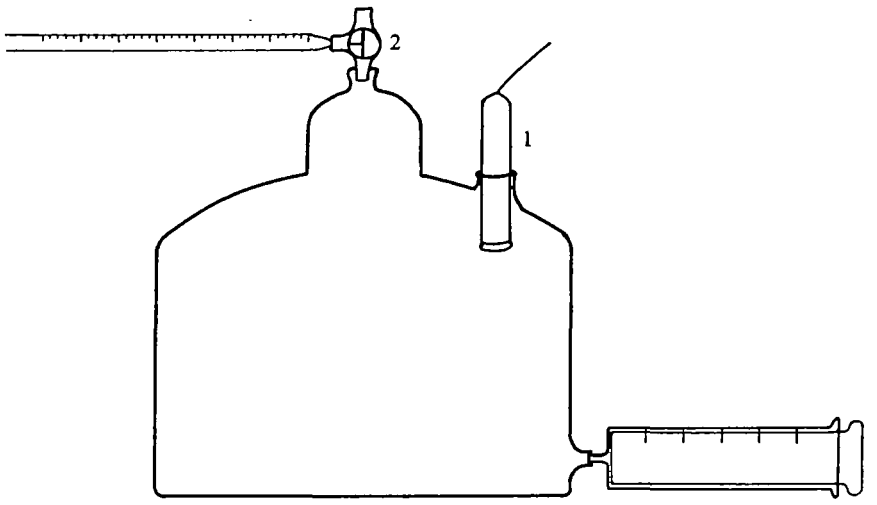

Fig. 1. Diagram of the vessel used in the experiments. 1, Oxygen electrode. 2, Stopcock for the withdrawal of gas samples.

out in an airtight glass vessel with the configuration shown in Fig. 1. This vessel had a total capacity of about 400 ml, and the small chamber on the top could hold a volume of 10 ml; gas samples could be taken from the chamber by means of a Hamilton syringe. When the total oxygen intake and carbon dioxide output were being measured, a frog was enclosed in the vessel with a small amount (30–40 ml) of water. Air samples were analysed for oxygen and carbon dioxide on a Fisher gas chromatograph every 60 min; it was verified in preliminary experiments in which liquid samples were also analysed that the liquid and the gaseous phases were in equilibrium under these conditions. The air and water in the chamber were completely renewed after each sample.

In order to separate the pulmonary and cutaneous functions the vessel was filled with water up to the lid in such a way that only a 10 ml air bubble was left in the upper chamber for the animal to breathe. This arrangement was chosen in order to minimize the area of the air/water interface, so that gaseous exchanges between the two media could be neglected if the gradients were kept small and the experimental periods were short. A 2 ml pipette filled with water and held horizontally was connected to the air bubble in the chamber. The withdrawal of water from the pipette balanced the excess of lung oxygen uptake over carbon dioxide output, keeping a constant gas pressure. Air samples were taken and analysed every 5 or 10 min, according to the breathing behaviour of the animal, and the air bubble was renewed after each sample. Water P_{O_2} was monitored by means of an oxygen electrode fitted into the chamber. Total water CO_2 was determined in a few cases on a Natelson microgasometer. Alternatively, as the CO_2 concentration in water was very low, practically at the limit of resolution of the instrument, total CO_2 was calculated from simultaneous determinations of water P_{CO_2} and pH, using the Henderson–Hasselbalch equation.

Oxygen uptake and CO_2 output by the skin were also determined during forced diving periods of 30 min in which no air was left in the chamber. This procedure did not seem to distress the animals and they resumed a normal pattern of diving and surfacing soon after they had access to air.

In a second group of experiments the P_{O_2} and P_{CO_2} of arterial blood were monitored over periods of emergence and diving. Frogs were anaesthetized by immersion in Tricaine (MS 222 Sandoz, 1·5 g/l) and a femoral artery was catheterized with a piece of nylon tube (Portex PP 10). The incision was closed, the animals were immobilized on a wire frame and left to recover in a glass tank, half immersed in water. The catheter was connected to oxygen and carbon dioxide electrodes contained in thermostatically controlled chambers. Their outputs were measured by a Radiometer Gas Monitor coupled to a pH meter 27. As the values of blood P_{CO_2} were too low to be read on the conventional CO_2 scale of the instrument, the CO_2 electrode was connected to the pH meter so that the readings could be obtained on the expanded pH scale. Calibration of the electrodes with gaseous mixtures having known CO_2 and O_2 contents was carried out daily. Total CO_2 concentration in the blood was determined on a Natelson micro-gasometer.

All the results are expressed as means ± S.D. unless otherwise specified. Student's *t* test was applied to evaluate the significance of differences between means; the *P* values are given in the text.

RESULTS

Total oxygen uptake and carbon dioxide output at rest were determined in 26 frogs which were kept for 60 min periods half immersed in water and breathing freely in the closed chamber. Under these conditions, oxygen uptake was 8·5 ± 2·2 ml. 100 g^{-1}. h^{-1} and free CO_2 output was 5·8 ± 1·3 ml. 100 g^{-1}. h^{-1}. CO_2 present as HCO_3^- in the water was not determined in these experiments. Pulmonary and cutaneous exchanges were evaluated separately in 32 animals, in a total of 47, 1 h periods, using the air-bubble technique. Lung oxygen uptake measured under these conditions was 7·6 ± 2·7 ml. 100 g^{-1}. h^{-1} while CO_2 release was only 2·5 ± 0·9 ml. 100 g^{-1}. h^{-1}. On the other hand, sampling of the water in which the animals were immersed showed that the cutaneous O_2 uptake was only 1·6 ± 0·04 ml. 100 g^{-1}. h^{-1} but a sizeable amount of CO_2 could be detected. A mean value of free CO_2 of 4·9 ± 0·9 ml. 100 g^{-1}. h^{-1} was calculated from the P_{CO_2} values found in 14 experiments, using a solubility coefficient of 1·105 at 22 °C (Edsall & Wyman, 1958). At the same time there was a decrease of the pH of the water, from initial values of 8·0–8·5 to 6·2–6·5. The amount of CO_2 present as HCO_3^- was evaluated using the Henderson–Hasselbalch equation ($pK' = 6·35$). The value of HCO_3^- thus found for the same experiments was 0·23 ± 0·08 m-moles. 100 g^{-1}. h^{-1}. These animals were also subjected to 30 min periods of forced diving, during which no access to air was permitted. The oxygen uptake through the skin was 2·7 ± 0·04 ml. 100 g^{-1}. h^{-1}, a significant increase ($P < 0·001$) over the values found when the animals has access to air. The decrease in pH of the water (down to 5·9–6·1 units) was also larger in most cases than in the conditions which allowed air breathing, as was the output of 'free' carbon dioxide (6·7 ± 1·1 ml. 100 g^{-1}. h^{-1}). The amount of bicarbonate in the water corresponded to 0·17 ± 0·06 m-moles. 100 g^{-1}. h^{-1}. Although the figures for both 'free' CO_2 and HCO_3^- measured during the enforced dive are significantly different from those obtained from the water when the animals were breathing at the surface, the total CO_2 output was similar in both conditions. The results of this group of experiments are summarized in Fig. 2.

On the next set of experiments we studied the time course of changes in blood gas

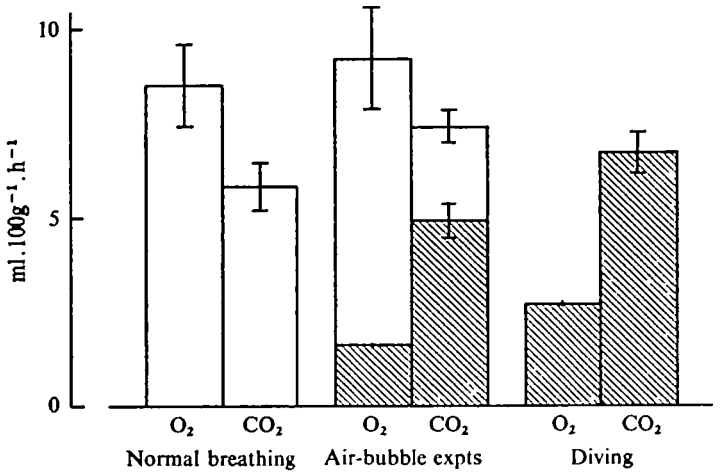

Fig. 2. Pulmonary and cutaneous gas exchanges. The first two columns represent total oxygen uptake and carbon dioxide output under 'normal breathing' conditions. The shadowed areas in the other columns represent skin oxygen uptake and free carbon dioxide output measured in the air-bubble experiments and during diving periods.

partial pressures under 'normal breathing' conditions and during diving periods. Blood P_{O_2} and P_{CO_2} were determined in ten animals in which a femoral artery was cannulated. Blood samples were withdrawn periodically to fill the electrode chambers and returned to the animal after reading the respective values. Typical values for arterial P_{O_2} and P_{CO_2} throughout such an experiment are shown in the plot of Fig. 3. P_{O_2} values varied between 65 and 90 mmHg during 'normal breathing' periods; this corresponds to total haemoglobin saturation, according to existing dissociation curves for amphibian blood (Lenfant & Johansen, 1967). After immersion, P_{O_2} values decreased rapidly within about 10 min to 10–20 mmHg, which represents an almost total depletion of the blood oxygen pool. Almost invariably the frogs were seen to breathe out soon after immersion; although we made no attempt to evaluate the lung oxygen store, we assumed that its contribution towards oxygen uptake during diving would be very small due to this fact.

The partial pressure of carbon dioxide was very low during 'normal breathing' periods (6–9 mmHg) but it increased progressively after diving to 14–20 mmHg. This increase was accompanied by an important lowering of blood pH values, from 8·0–7·8 pH units to 7·3–7·2.

The rise of P_{CO_2} was somewhat surprising, as we had seen in the earlier experiments that the elimination of CO_2 through the skin was at least as large during diving periods as when the animals were at the surface and that lungs had a relatively minor role in CO_2 disposal. Furthermore, the O_2 uptake during immersion is minimal and the O_2 reserve is too small to keep the usual levels of aerobic metabolism going for more than 5 min. Thus a decrease both of CO_2 production and of the size of the CO_2 pool during prolonged diving might be predicted. In order to test this hypothesis determinations of total CO_2 in the arterial blood of a group of animals were carried out, and the values found during breathing periods were compared with those found after 20–30 min of immersion (Table 1). In fact we verified that the total CO_2 concentration in the blood

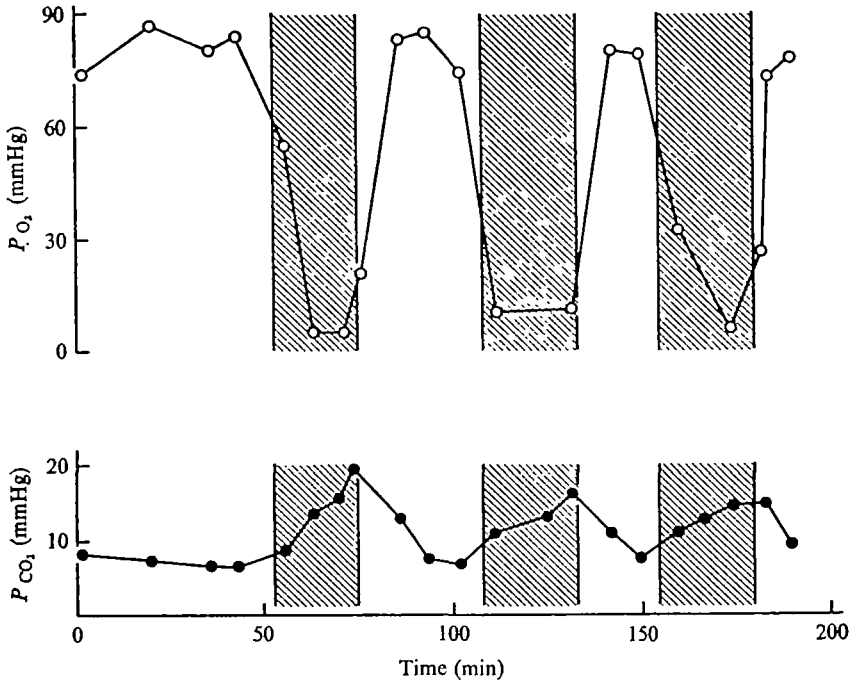

Fig. 3. Partial pressures of oxygen and carbon dioxide in arterial blood. The shadowed areas represent immersion periods.

Table 1. *Blood gases, pH and total CO_2 in frogs before, during and after immersion periods*

(The blood samples in the 'after diving' column were taken 30 min after emergence in Expts 1–5 and 60–90 min after emergence in Expts 6–10.)

	P_{O_2} (mmHg)			P_{CO_2} (mmHg)			m-moles/l CO_2 (total)			pH		
Expts	Before diving	Diving	After diving	Before diving	Diving	After diving	Before diving	Diving	After diving	Before diving	Diving	After diving
1	80	10	89	6·7	18·6	7·7	14·0	8·7	—	7·83	7·36	7·51
2	98	—	52	7·1	20·0	7·6	16·9	9·5	11·3	8·00	7·28	7·71
3	100	10	90	9·3	15·5	9·3	11·5	9·0	7·0	7·80	7·41	7·54
4	103	5	93	10·0	18·9	10·4	9·0	8·5	9·0	7·64	7·31	7·54
5	100	5	90	6·5	15·3	8·4	12·2	8·3	6·6	7·90	7·27	7·57
6	104	9	96	7·2	19·2	8·9	13·0	7·7	10·6	7·92	7·27	7·69
7	96	5	87	8·9	20·8	7·7	12·6	9·8	—	7·74	7·18	7·46
8	72	5	78	8·4	18·0	8·0	12·3	10·7	10·7	7·82	7·43	7·82
9	80	5	82	7·6	16·0	7·3	13·0	9·3	11·0	7·91	7·47	7·69
10	104	3	97	7·1	12·7	7·5	10·6	9·2	14·5	7·82	7·56	7·91

decreased significantly during an immersion period, although the P_{CO_2} values were about twice as large as the control values. Furthermore, the recovery of total CO_2 levels similar to the control values was very slow, specially after long immersion periods, although normal P_{CO_2} values were found 5 or 10 min after emergence. This is also shown in Table 1, where recovery values found between 30 and 90 min after an immersion period are tabulated.

DISCUSSION

The rates of oxygen consumption by anuran amphibia have a wide range of variation according to the species and are also strongly influenced by the temperature at which the determinations are made (Fromm & Johnson, 1955; Jones, 1972*a*). Although we made no attempt to acclimate the animals for prolonged periods to the laboratory conditions, values of total oxygen uptake at rest for *Rana ridibunda* found in the present work lay between the values given by Hutchison, Whitford & Kohl (1968) for *Rana pipiens* acclimated to 15 and 25 °C, and are also within the range given by Jones (1967) for different species of frogs acclimated to 17 °C.

The predominance of the lung respiratory surface for oxygen uptake described in other amphibians (Hutchison *et al.* 1968; Vinegar & Hutchison, 1965; Whitford & Hutchison, 1963) was confirmed in our experiments. When the pulmonary and cutaneous respiratory functions were evaluated separately, it was found that about 80% of total oxygen uptake was achieved through the lungs and only the remaining 20% was absorbed through the skin (the frogs were almost completely immersed in water during these determinations; the proportion of oxygen absorbed through the skin might have been higher in an air-filled respirometer). The increase of the skin oxygen uptake during apnoeic periods, although statistically significant, was obviously too small to support the normal metabolic demands of the animals. In fact, the skin uptake during immersion corresponded to 35%, approximately, of the total oxygen uptake during breathing periods. This was reflected in the extremely low values for the partial pressure of oxygen in the arterial blood determined only a few minutes after immersion, which showed an almost complete depletion of the blood oxygen store. This store may be roughly estimated as 0·7 ml for a 100 g animal, based on the amphibian oxyhaemoglobin dissociation curves published by Lenfant & Johansen (1967) and assuming a total blood volume of 8% of the body weight (Jones, 1972*b*). This value corresponds to the total oxygen consumption in 5 min under normal breathing conditions. Even admitting the uptake of some oxygen from the lungs after diving, the survival of the animals for much longer periods of apnoea has to be attributed partly to other factors, perhaps to an overall reduction in metabolism, which has been confirmed in *Rana esculenta* by a fall in heat production (Jones, 1972*b*). In addition, anaerobic processes may also make an important contribution to the total metabolism during diving periods (Rose & Drotman, 1967). The pulmonary output of carbon dioxide was only about one third of the pulmonary oxygen uptake, confirming the fact that most of the metabolic carbon dioxide production is disposed of through the skin. The fraction of free CO_2 measured in the water where the animals were immersed, plus the CO_2 present as bicarbonate, is four to five times as much as the pulmonary fraction. Actually, the total CO_2 output so evaluated is higher than the total oxygen uptake measured in the same periods. A R.Q. greater than 1 has been repeatedly found in amphibia, specially in animals which had been kept in cold chambers and starved, as in our experiments (Fromm & Johnson, 1955). However, it is very probable that at least part of this excess of total CO_2 does not arise from respiratory exchanges but from the skin glandular secretions, which have been shown to be rich in bicarbonate (Friedman, Laprade, Aiyawar & Huf, 1967).

The most striking feature in these experiments was the finding that the skin CO_2

output did not seem to decrease during diving although the aerobic metabolism must have been substantially reduced, as we have seen before. Important modifications of the acid-base state of the blood did occur, however, and these were expressed as large decreases in both pH value and total CO_2 content accompanied by an increase in the partial pressure of CO_2. The CO_2 given off during a dive could, in part, be attributed to the change in CO_2 content of the body. The loss of blood CO_2 after 30 min of immersion was approximately 3 μmoles/ml (Table 1). Assuming that there is an extracellular space of 30% body weight in equilibrium with the blood the total loss in a 100 g animal would be about 0·1 m-moles in 30 min. This figure may be compared with the actual CO_2 output for the same period, which was 0·15 m-moles as calculated from our results. The difference between the two figures could be accounted for by the reduced level of oxidative metabolism still going on. A switch to anaerobic metabolism when the animals were deprived of oxygen would result in the passage of organic acids to the plasma, thus producing a metabolic acidosis with a fall in pH and a large decrease in blood HCO_3^-. The situation is further complicated by the loss of the lungs as a site for CO_2 elimination, leading to increased P_{CO_2} in the blood, a larger gradient across the skin and substantially the same level of CO_2 elimination as was formerly achieved by both skin and lungs together.

These changes would thus tend towards a new acid-base relationship in which the total CO_2 in the blood is very much reduced and an acidosis with both metabolic (organic acids) and respiratory (increased P_{CO_2}) components is seen. So far we have made no attempt to measure organic acids in the blood of frogs, but an increase in lactic acid has been described in toads following prolonged submergence (Leivestad, 1960). The rapid fall in P_{CO_2} which is seen when the animal emerges and begins to breathe must be due to the lungs once more becoming effective in CO_2 elimination. The respiratory acidosis was thus reversed quite quickly and the pH went up. Complete recovery of total CO_2 and pH levels was delayed, however, and this we attribute to the slow removal of organic acids from the blood.

SUMMARY

1. The respiratory exchanges through the lungs and skin of frogs and the time courses of blood gas concentrations were studied during emergence and diving periods.

2. Most of the total oxygen uptake is carried out through the lungs. The partial pressure of oxygen in arterial blood falls to very low levels a few minutes after diving, showing that the cutaneous respiratory surface cannot compensate for the lack of lung respiration.

3. Most of the metabolic carbon dioxide is disposed of through the skin. Although the skin output is maintained through diving periods, there is an important rise in the partial pressure of carbon dioxide in blood following submergence. However, the total concentration of CO_2 in the blood decreases, as does the blood pH value.

4. This phenomenon is probably the result of a metabolic acidosis due to the switching on of anaerobic processes during diving periods.

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