

CARBON DIOXIDE EXCHANGE AND ITS EFFECTS ON pH AND BICARBONATE EQUILIBRIA IN THE BLOOD OF THE AMPHIBIAN, *XENOPUS LAEVIS*

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SUMMARY

1. The elimination of CO_2 through the lungs and skin of *Xenopus laevis* and its distribution between plasma and erythrocytes were determined during breathing and submergence periods.

2. CO_2 output does not decrease during submergence, as lack of lung ventilation is compensated by an increase in cutaneous exchange. The total CO_2 carried in the blood decreases after a 30 min dive, but the fraction carried in red blood cells increases substantially, due to an increase in the haematocrit values. This increase is related to a decrease in plasma volume during diving.

3. During short dives the blood shows changes characteristic of respiratory acidosis but longer dives result in a combination of respiratory and metabolic acidosis.

INTRODUCTION

The breathing pattern of *Xenopus laevis* is usually characterized by long intervals of apnoea during diving punctuated by short outbursts of lung ventilation. It has been shown that this type of intermittent breathing is accompanied by fluctuations in the partial pressures of O_2 (P_{O_2}) and CO_2 (P_{CO_2}) in the blood (Emilio & Shelton, 1974). A decrease in P_{O_2} during diving is explained by the observation that the lung oxygen store is rapidly depleted during the first minutes of submersion (Jones, 1967) and that the uptake of oxygen through the skin does not increase substantially when the animal is submerged. The changes in arterial P_{CO_2} , however, are not so straightforwardly explained. It has been shown in anurans that a high percentage of CO_2 is eliminated through the skin (Foxon, 1964) and this function should not be seriously impaired by submersion. In addition, a switch to anaerobic metabolic processes has been demonstrated in amphibia during diving periods (Rose & Drotman, 1967) so that cellular production of CO_2 ought to decrease.

However, other factors contribute to the complexity of the situation. For example,

it is known that there is a considerable fluctuation of pulmocutaneous blood flow in *Xenopus*, related to the periods of ventilation (Shelton, 1970), which may affect the degree of perfusion of the skin and its functions as a gas exchanger. Moreover, a modification of the overall metabolic processes may also give rise to a change in the acid-base balance of the blood towards a situation of metabolic acidosis. Under these conditions the parameters of the $\text{HCO}_3^-/\text{CO}_2$ equilibrium in red blood cells and plasma would be altered (Emilio, 1974), causing the relationship between blood gases and environment to vary in a complex way.

Very little is known about the properties of amphibian blood with respect to the carriage of CO_2 in cells and plasma, and so the present experiments were designed to examine the effects of diving on CO_2 equilibria in the blood. Before changes in these equilibria could be understood it was necessary to examine the gas exchange relationships in breathing and diving toads.

METHODS

Measurements of the rates of O_2 and CO_2 elimination through the lungs and skin were carried out on *X. laevis* weighing between 37 and 45 g. Each animal was confined in an air-tight glass vessel with a capacity of 380 ml and filled with 5 mM sodium bicarbonate solution. A small chamber which could hold 10 ml of air formed an extension to the upper, central, region of the main chamber. Gas samples of 500 μl were taken from this upper compartment with a Hamilton syringe, and analysed chromatographically for O_2 and CO_2 on a Fisher Gas Partitioner (model 25V). A Radiometer oxygen electrode was introduced in the main chamber to monitor continuously the decrease of P_{O_2} in the solution. Cutaneous CO_2 elimination was determined by titrating the bicarbonate solution continuously during the experiment with 0.2 N sodium hydroxide (Gotlieb & Jackson, 1976). The pH of the solution was measured with a combined pH electrode (GK 2320 C) and the titration carried out to a pH of 8.20 using an automatic system (Radiometer TTT11 and titrigraph SBR 2C), the output of the syringe burette leading directly to the animal chamber. Under these conditions the P_{CO_2} in the solution bathing the animal remained at a constant level of about 1 mmHg. The volume of CO_2 produced was calculated from the volume of NaOH added. The sodium bicarbonate solution in the experimental chamber was renewed every 15–20 min so that the decrease of P_{O_2} in the medium was less than 30 mmHg. Gas samples were taken every 5–7 min and the air bubble completely renewed after each sample. These determinations were continued over a period of 45–60 min, so that 3–4 measurements of O_2 consumption and CO_2 liberation through the skin and 9–12 measurements of gas exchange through the lungs were made during this first part of the experiment. Then the solution was substituted again and the upper chamber filled so that the animal had no access to air for a further period of 15 min. Oxygen uptake and CO_2 output through the skin only were determined during this period, using the same methods of measurement as described above. The results were averaged for 1 h and 100 g body weight.

Adult female *X. laevis* (90–120 g body weight) were used for the experiments in which measurements of CO_2 content, pH and gas tension levels were made on blood samples. The animals were anaesthetized by immersion in tricaine methane sul

phonate (MS222 Sandoz, 1.5 g/l) and a sciatic artery was cannulated with a polythene tube (Portex PP60). Heparin (10 i.u./100 g body weight) was injected and the cannula was filled with heparinized saline (2 i.u./ml). The incision was closed and the animals were left to recover in a small tank, in a temperature-controlled room (25 °C), for about 24 h. An initial blood sample of 250 µl was collected in five or six heparinized capillary tubes when the animal was breathing at the surface. Then a lid was lowered to the water surface, so that the animal had no access to air for periods of 10 min in one set of experiments and 30 min in a second. A further blood sample was taken at the end of these periods and the lid was then taken out to allow the animal to reassume its normal breathing behaviour. Further blood samples were then taken approximately 1 and 4 h after the forced dive, generally during a breathing spell. Blood pH, P_{CO_2} and P_{O_2} were determined in the samples by means of electrodes incorporated in a Radiometer BMS 2 apparatus. Haematocrits were measured in each sample using an International microcapillary centrifuge (model MB) at 15 000 rev/min for 5 min. The red blood cells of *Xenopus* are large and packing during centrifugation might not be complete. The average amount of plasma trapped among the erythrocytes was, therefore, determined in 15 samples using ⁶⁰Co (EDTA) as an extracellular tracer (Brading & Jones, 1969). 5 µl of a 1 mM-Co (EDTA) solution with a trace of ⁶⁰Co were added to 50 µl of blood, so that the final concentration of the carrier was 1 mM. The blood was centrifuged in haematocrit tubes and the radioactivity of the plasma and red blood cells was determined. The values thus obtained for trapped plasma fell between 1 and 3%, and no correction was, therefore, made in subsequent calculations.

Total CO₂ in whole blood and plasma was determined, in most cases, by the method of Cameron (1971); in some experiments a Natelson microgasometer was used, the two techniques giving identical results. Blood or plasma samples of 20–30 µl were utilized for the Cameron chamber. The sample was introduced into a small cuvette containing 0.1 N-HCl and the increase in P_{CO_2} was measured by a Radiometer E5037 electrode. The system was kept at 35 °C, to shorten the response time of the electrode, and it was calibrated each day with standard bicarbonate solutions.

Red blood cell volume was determined in a group of five adult females (90–100 g body weight). Red blood cells from a donor were labelled with ⁵¹Cr (Jones, 1970), resuspended in plasma and injected in the circulation, the exact amount being determined by weighing the syringe before and after the injection. Blood samples were taken 20 and 40 min after the injection; then, the animal was forced to remain submerged for 30 min, and another blood sample was taken. A final sample was withdrawn 60 min after the animal had been allowed to the surface. Haematocrits and red blood cell counts were determined at each sampling period. A Picker-Pace I Gamma System was used to determine the radioactivity of the samples, and the red blood cell volume was calculated. Total blood volume and plasma volume were assessed from the haematocrit. Erythrocyte counts were carried out using a Neubauer Haemocytometer.

Table 1. *Oxygen consumption and carbon dioxide output in X. laevis (ml. 100 g⁻¹.h⁻¹)*

	Lungs		Skin		Total	
	O ₂	CO ₂	O ₂	CO ₂	O ₂	CO ₂
Access to air (n = 9)						
Mean	6.1	1.1	2.5	6.9	8.6	8.0
S.E.M.	0.34	0.17	0.14	0.98	—	—
No access to air (n = 13)						
Mean	—	—	3.9	7.3	—	—
S.E.M.	—	—	0.20	0.54	—	—

RESULTS

(A) *Oxygen uptake and carbon dioxide output through lungs and skin*

The O₂ uptake through the lungs was more than twice as large as the uptake through the skin when the animals had access to air (Table 1). When the access to air was cut off the uptake through the skin increased, but it was still less than half the total value found during air-breathing. On the other hand, less than 15% of the CO₂ was eliminated through the lungs, and the CO₂ output through the skin when the animals were not allowed to breathe was practically identical to the total output through the lungs and skin during breathing periods. It should be emphasized that the results shown for the so called breathing animals do not represent gas exchange during continuous ventilation. The animals concerned were free to surface and dive and, in fact, spent a considerable proportion of the time submerged. Thus the breathing results in Table 1 are a combination of periods of submersion when CO₂ output may have exceeded O₂ uptake (as shown in the diving results in the same Table) with shorter periods of lung ventilation when the reverse was probably the case.

The total O₂ uptake found in these experiments is significantly higher than the value found in earlier determinations (4.8 ml O₂ 100 g⁻¹.h⁻¹: Emilio & Shelton, 1974) although the distribution between skin and lungs is approximately the same. The animals used in the present experiments were smaller and younger, and the room temperature was 25 °C instead of 20 °C as in the previous work but these factors were found to be relatively unimportant compared with the influence of chamber size and shape. In the present work the animals had to exert a considerable muscular activity to reach the air bubble at the top of the chamber, whereas in the former experiments oxygen consumption was measured in a chamber where the animals had an easy access to air. This latter chamber could not be used in the present work because it was not designed to permit simultaneous measurements of the CO₂ output through skin and lungs.

(B) *Effects of diving on the carbon dioxide distribution in the blood*

Although it is usual for undisturbed *Xenopus* to stay motionless at the bottom of a tank for periods of 20–30 min, all animals subjected to surgery tend to breathe more frequently; thus, when the lid of the tank was lowered to the water level the cannulated animals tried to breathe within the first 10 min and spent most of the remaining time moving about the tank, probably in attempts to reach the surface. In the longer dives

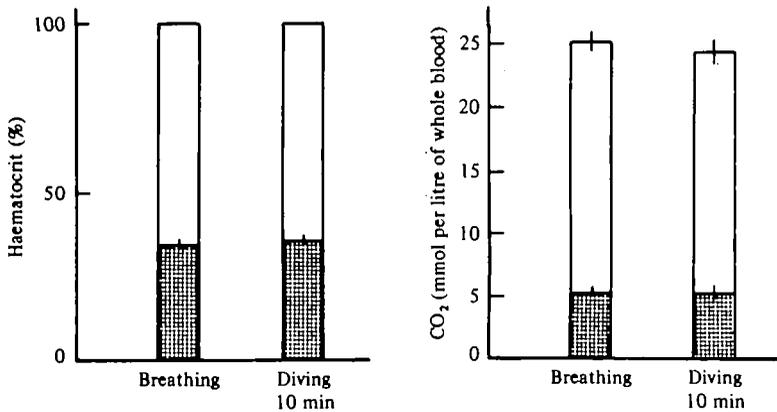


Fig. 1. Haematocrit values and distribution of CO₂ between plasma and erythrocytes during breathing periods and after a 10 min dive. The shadowed areas represent erythrocytes. Means and s.e.m. of nine experiments.

Table 2. Determinations of pH and respiratory gas values in blood samples taken from *Xenopus* before and after a 10 min submersion

Conditions	Blood CO ₂ (mM)	Plasma CO ₂ (mM)	pH	P _{CO₂} (mmHg)	P _{O₂} (mmHg)
Breathing (n = 14)					
Mean	25.2	31.3	7.69	13.0	103.0
S.E.M.	0.92	0.65		0.7	6.1
Diving (n = 9)					
Mean	24.3	31.9	7.55	17.0	32.0
S.E.M.	1.03	0.46		0.5	2.5

there was therefore considerable muscular activity, the metabolic effects of which must have an important influence on results obtained. So far no attempt has been made to measure any of these effects, such as the accumulation of end products of anaerobiosis, like lactate, which might have influenced the pH state of the blood.

The results obtained before and after submitting the animals to short (10 min) diving periods are summarized in Table 2. Changes in blood gas pressures and pH are evident, but no significant differences (at the 5% level) were found between breathing and diving states in the total CO₂ content of the whole blood, its partition between plasma and red blood cells and the average haematocrit values (Fig. 1).

A different pattern emerged from the experiments with forced dives of 30 min duration. A striking increase was seen in the haematocrit values of blood samples obtained after a long dive, as compared with the haematocrits of oxygenated samples (Fig. 2). The determination of total CO₂ concentration in whole blood and plasma showed that both values decreased at the end of a 30 min dive (Table 3). However, the amount of CO₂ carried in the red blood cells, calculated as the difference between the amount in whole blood and the amount contained in the plasma volume found in haematocrit measurements, was significantly increased (Fig. 3). The fraction of total CO₂ carried in the r.b.c. of the breathing animals in the initial part of the

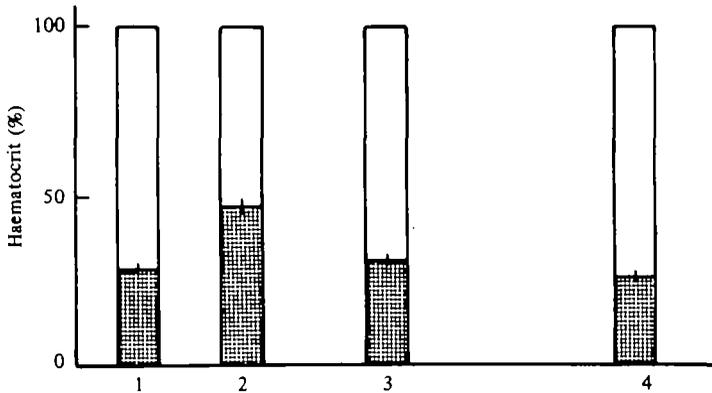


Fig. 2. Haematocrit values determined in experiments where the animals were subjected to 30 min submersion. (1) Access to surface for lung ventilation; (2) end of 30 min dive; (3) end of 1 h recovery with access to surface; (4) end of 4 h recovery with access to surface. Means and S.E.M. of ten experiments.

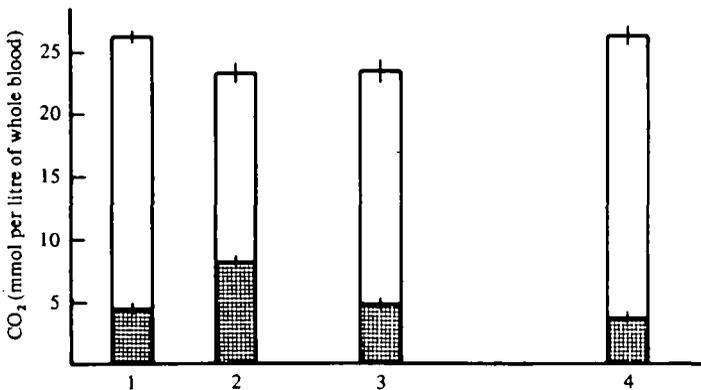


Fig. 3. Total CO₂ content of blood and its partition between plasma and erythrocytes, before and after a 30 min submersion. (1) Access to surface for lung ventilation; (2) end of 30 min dive; (3) end of 1 h recovery with access to surface; (4) end of 4 h recovery with access to surface. Means and S.E.M. of ten experiments.

experiment was 15.7%. After a 30 min dive this fraction represented 34.8% of the total CO₂. In a total of ten experiments, we calculated that 1 ml of oxygenated blood contained 21.7 ± 0.92 μmol of CO₂ in the plasma (means \pm S.E.M.) and 4.7 ± 0.41 μmol in the red blood cells. After a 30 min dive, however, total blood CO₂ was only 23.3 ± 0.92 μmol , and a higher fraction of this total was carried by the erythrocytes (15.1 ± 1.03 μmol in plasma and 8.1 ± 0.41 μmol in the cells in 1 ml of blood). The changes in blood P_{CO_2} and pH were more marked in these experiments than in the ones where the animals were subjected to shorter anoxic periods.

The sampling of blood 1 h after a forced 30 min dive showed that the blood gas values and haematocrit were back to normal, but the total CO₂ concentrations in whole blood and plasma were still low (Table 3 and Fig. 2). The calculated partition of CO₂ between plasma and erythrocytes showed that the contents in cells was not

Table 3. Total CO₂ in whole blood and plasma, blood pH, P_{CO₂} and P_{O₂} of *X. laevis* before and after a 30 min submersion

	Whole blood CO ₂ (mM)	Plasma CO ₂ (mM)	pH	P _{CO₂} (mmHg)	P _{O₂} (mmHg)
(1) Access to surface. Breathing preceding dive (n = 10)					
Mean	26.2	30.4	7.71	13.5	78.8
S.E.M.	0.57	0.92		0.34	4.6
(2) End of 30 min dive (n = 10)					
Mean	23.3	28.0	7.49	20.0	24.6
S.E.M.	0.92	1.22		0.85	1.8
(3) 1 h after access to surface. Breathing following dive (n = 8)					
Mean	23.4	26.1	7.65	13.0	87.0
S.E.M.	1.03	0.67		0.78	6.5
(4) 4 h after access to surface. Breathing following dive (n = 7)					
Mean	26.3	29.9	7.71	15.0	84.0
S.E.M.	0.88	0.73		0.45	6.8

different from what was found before the submergence, but the plasma value was still significantly lower (Fig. 3). Four hours after the forced diving period the values of all the variables studied were identical to the values determined before the dive (Table 3 and Figs. 2 and 3).

The increase in haematocrit values during a long dive could have been due to a swelling of the cells or to an increase in the cell count per mm³, either through an increase of the number of cells in circulation or through a decrease in plasma volume. Experiments were carried out to assess red cell volume using erythrocytes labelled with ⁵¹Cr. Total blood volume was calculated from the haematocrit. Two samples were taken while the animals were swimming freely, during breathing spells, and the results were averaged. Another sample was taken at the end of a 30 min forced submersion and a final one 60 min later.

The results obtained from five such experiments are expressed in Table 4. There is a significant increase (at the 0.01 level) of the haematocrit values and the erythrocyte counts of the blood taken at the end of the submersion period. Total red blood cell volume does not vary significantly, and plasma volume and total blood volume decrease sharply. One hour after emergence none of the measured values is significantly different from the initial ones.

The relationship between red blood cell count and haematocrit is: 204450 r.b.c. per 10% Ht in the initial period, 192350 r.b.c. per 10% Ht after 30 min submergence, and 190000 per 10% Ht on recovery. Although these figures are not significantly different at the 0.01 level they may reflect a certain amount of swelling of the erythrocytes during long dives.

DISCUSSION

The experiments on gas exchange reported in this paper indicate that rates of transfer of the two respiratory gases are differently affected by the alternation of lung ventilation and apnoea. The pattern of O₂ uptake was the same as that found in earlier experiments (Emilio & Shelton, 1974), though the actual values for O₂

Table 4. *Determinations of haematocrit, erythrocyte count and erythrocyte volume in blood samples, taken from adult Xenopus (n = 5, body weight 90-100 g)*

(Determinations taken at the following times: (A) during breathing spells before submersion (average of two determinations for each animal); (B) at the end of a 30 min forced submersion; (C) 1 h after access to air. Plasma and total blood volumes calculated from the haematocrit and r.b.c. volume.)

Blood samples		Haematocrit (%)	Erythrocyte count (cells/mm ³)	(ml/100 g body weight)		
				R.b.c.	Plasma	Total blood
A	Mean	29.6	605200	4.0	9.4	13.4
	S.E.M.	3.1	31200	0.29	0.82	0.97
B	Mean	42.5	817500	3.7	5.0	8.7
	S.E.M.	0.70	27000	0.39	0.47	0.76
C	Mean	31.1	590900	3.7	8.0	11.7
	S.E.M.	0.50	20700	0.17	0.36	0.48
<i>t</i> test (<i>P</i>)						
A-B		< 0.01	< 0.001	> 0.4	< 0.01	< 0.01
A-C		> 0.5	> 0.5	> 0.2	> 0.1	> 0.1

consumption were a little higher because of changes in design of the experimental chamber. When the animal dived the total O₂ consumption fell considerably, even though O₂ uptake through the skin increased a little above the values recorded at the surface. As the stores of O₂ in lungs, blood and tissues are small, the metabolizing cells must be subject to continuous fluctuations in O₂ supply. During a dive this supply is insufficient to support the full aerobic demands of metabolism (Jones, 1972) and survival depends on a switch to anaerobiosis or an overall reduction in metabolism (Rose & Drotman, 1967; Jones, 1972; Emilio & Shelton, 1974; D'Eon, Boutilier & Toews, 1978). On the other hand, transfer of CO₂ from animal to medium, for which the skin is of greater importance than the lungs, was not significantly reduced when the animal dived. The relationships are obviously complex.

Though the time period over which the determinations were made was relatively short, the overall relationships between CO₂ and O₂ exchange through both lungs and skin are probably those shown for animals which had access to air in Table 1. The average RQ for such animals is about 0.9, but this must be made up of much higher values during diving periods (Table 1), and very much lower values during the brief periods of lung ventilation. There is thus a time dependence in exchange relationships between the two gases related to the following factors: (1) the changing metabolic rate and the relative importance of aerobiosis and anaerobiosis, (2) the ventilation, perfusion and diffusion characteristics of skin and lungs and (3) the effects of constantly changing gas tensions in tissues and blood on the quantities of gases transferred and stored. The relatively constant output of CO₂ from the animals during the present experiments in face of a fluctuating metabolic production of the gas can be accounted for in terms of these factors.

The changes of the acid-base status of the blood of *Xenopus* after a long period of submersion are similar to those previously found in *Rana ridibunda* (Emilio, 1974). In both species a decrease was observed in the total CO₂ content and the pH of the blood, together with an increase in *P*_{CO₂}. The *P*_{CO₂} increase must facilitate diffusion of

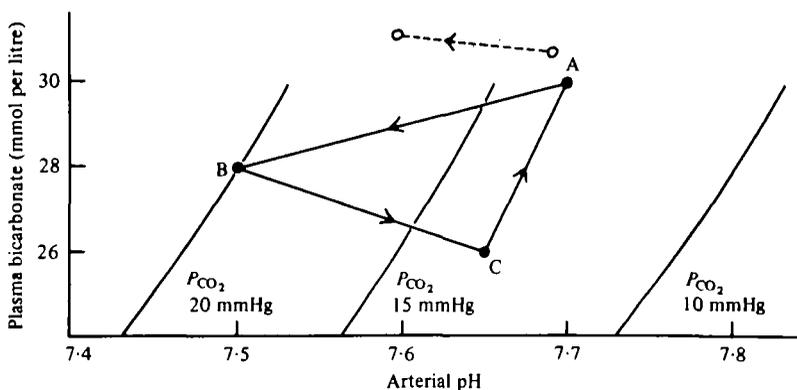


Fig. 4. Diagram of changes occurring in the $\text{HCO}_3^-/\text{CO}_2$ system when the animals were subjected to 30 min dives. Point A: arterial point when animals had access to air and were actively breathing. Point B: after 30 min forced dive. Point C: 1 h after dive was terminated and the animals allowed access to air. The dotted line shows the changes which occurred after 10 min dive.

CO₂ through the skin, though the relationship is almost certainly complicated by changes in skin perfusion during a dive. Unfortunately such changes are not at present fully understood. Since CO₂ production decreases during a dive, the high P_{CO_2} levels found in this situation must be the result of absence of lung ventilation and the establishment of a certain degree of metabolic acidosis through the triggering of anaerobic processes during submersion (Rose & Drotman, 1967). The elimination of CO₂ at the same rate as during emergence periods thus results in a decrease in the total CO₂ pool of the animal. This is reflected in the low value for total CO₂ content in the blood at the end of a dive and during the early period of recovery.

A similar pattern of acid-base changes has been described in freshwater turtles subjected to long experimental dives (Jackson & Silverblatt, 1974). These animals have a very low plasma bicarbonate after a dive, which was also attributed to loss of carbon dioxide through the body surface. As Jackson and Silverblatt point out, most diving animals such as beavers, ducks, and alligators show an increase in blood CO₂ content during a dive. In turtles, and probably to an even greater extent in *Xenopus*, the situation is different because of the conditions for elimination of CO₂ through the body surface.

The sequence of events in our experiments, with respect to changes in the $\text{HCO}_3^-/\text{CO}_2$ system and the pH of the blood is summarized in Fig. 4, using data from Tables 2 and 3. Point A represents the equilibrium state during normal lung ventilation. During a long period of submersion, there is a shift towards point B with a loss of plasma bicarbonate reflecting a depletion of the total CO₂ pool, a marked decrease in pH and an increase in P_{CO_2} . When the animals are able to breathe again, there is a rapid fall of P_{CO_2} as CO₂ is removed through the lungs during ventilation. During this period the plasma pH increases and bicarbonate falls along an *in vivo* buffer line to point C. Finally there is a slow restoration of the HCO_3^- pool, a process which may take some time to accomplish depending on the extent of the metabolic acidosis. Fig. 4 also shows the trend of changes during a short (10 min) dive, during which there is no depletion of the HCO_3^- pool. In this case the changes are typical of a respiratory

acidosis uncomplicated by metabolic effects, with high P_{CO_2} , low pH, and slightly increased bicarbonate.

Under normal breathing conditions with free access to air, or after short dives, about 85% of the total CO_2 in blood is carried in the plasma. After a 30 min dive, however, this proportion is reduced to a value of about 65% and, though the total CO_2 in the blood decreases, there is an absolute increase in the amount carried by the erythrocytes. This is due largely to a relative increase in the cell volume, as reflected by the haematocrit values, more than through an increase in CO_2 concentration in the cells. A higher erythrocyte volume in venous blood as compared to arterial blood is found in mammals (Roughton, 1964), although the phenomenon is not so marked as the changes found for *Xenopus*.

In the present study, the haematocrit of arterial blood went up from 29% to 46% in one series of experiments (Fig. 2) and from 29.6% to 42.5% in another (Table 4) during the course of a 30 min dive. As the total volume of erythrocytes did not change significantly (as shown in the experiments with ^{51}Cr -labelled cells) we must conclude that under the extreme hypoxic conditions established during a forced submergence period of 30 min there is a decrease in plasma volume and in total blood volume. This fall in blood volume may be explained in terms of water movement into the cells of tissue such as muscle, particularly when they are active during hypoxia. Other possibilities such as a modification of the total water balance of the animals subjected to our experimental conditions cannot at the moment be excluded.

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