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THE EFFECTS OF FORCED AND VOLUNTARY DIVING ON VENTILATION, BLOOD GASES AND pH IN THE AQUATIC AMPHIBIAN, *XENOPUS LAEVIS*

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SUMMARY

1. Pre- and post-dive breathing patterns, blood oxygenation and acid–base balance have been examined in voluntarily and forcibly submerged *Xenopus laevis*.

2. Enforced 30-min dives led to a large acidosis with both respiratory (CO_2) and metabolic (lactic acid) components. Complete recovery of the arterial blood variables after such dives took more than 4 h.

3. Lung ventilation (measured by a pneumotachograph) following enforced dives was always markedly elevated compared with levels either before or after voluntary dives of the same duration.

4. In undisturbed *Xenopus*, diving freely for periods of 30 min or more, there was no accumulation of lactic acid and the fall in blood oxygen, increase in CO_2 and the associated respiratory acidosis were all corrected within the first few breaths upon surfacing.

5. The evidence presented here leads us to conclude that anaerobiosis is unimportant during voluntary dives, even when these are of considerable duration.

INTRODUCTION

Most cardiorespiratory studies on diving in anuran amphibians have involved either submersion of restrained animals or closing off access to the surface for freely moving individuals. Periods of breath holding under these circumstances result in a well developed diving bradycardia, together with a rapid decline of the blood oxygen store and a combined respiratory and metabolic acidosis (Lenfant & Johansen, 1967; Jones, 1972; Emilio, 1974; Lillo, 1978; Emilio & Shelton, 1980). This has been taken to suggest that anaerobic pathways make a significant contribution to the overall energy metabolism of the submerged animal. Anaerobiosis is indeed a major constituent of the activity metabolism of many amphibians as shown by the increase of lactate in the blood and tissues during exercise (Bennett, 1978). After exercise, the restoration of the lactate concentrations and HCO_3^-/CO_2 equilibria in the blood

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takes several hours (Hutchison & Miller, 1979*a,b*; McDonald, Boutilier & Toews, 1980; Boutilier, McDonald & Toews, 1980). Recovery from enforced anaerobic dives in *Xenopus* appears to be equally time-consuming (Emilio & Shelton, 1980), and this must have some impact on the frequency and duration of subsequent dives.

There is little evidence, however, to indicate that anaerobic metabolism is of major importance during periods of unrestricted voluntary diving. For example, voluntary dives in both *Amphiuma* and *Xenopus* are terminated before oxygen stores become fully depleted (Toews, Shelton & Randall, 1971; Emilio & Shelton, 1974; Boutilier, 1984). In addition, although these animals will voluntarily dive for up to an hour or more, they do not show a post-dive hyperpnoea. There is reason to doubt, therefore, that anaerobiosis plays a significant role in the diving metabolism of unrestrained amphibians during periods of voluntary submergence. In the present paper, we examine this hypothesis by comparing the blood acid–base and oxygenation characteristics together with pre- and post-dive breathing patterns in voluntarily and forcibly submerged *Xenopus laevis*.

MATERIALS AND METHODS

Xenopus laevis (95–153 g) were obtained from a commercial supplier and held in the laboratory at 25 °C for several months before the experiments. Each animal was anaesthetized by immersion in a 0·06 % solution of tricaine methane sulphonate (MS-222, buffered to pH 7·0), and a cannula was chronically implanted into the femoral artery as described by Boutilier (1984).

Two series of experiments were carried out, both of which continuously monitored ventilation using a pneumotachograph apparatus (see Boutilier, 1984). Briefly, air flow out of a small cylindrical breathing chamber (positioned at the water surface) was passed through a pneumotachograph screen. Pressures on either side of the screen were detected by a differential pressure transducer (Hewlett-Packard Model 270) whose output was stored on an instrumentation tape recorder (Racal Store 4) and recorded on a Medelec For-4 oscillograph. Air flow rates were later integrated to give ventilation volumes. The output from the transducer was linearly related to flow over the range of flow rates encountered. Below the breathing hole the animal was enclosed in an aerated volume of water (25 ± 0·5 °C) large enough to permit free movements for voluntary diving and surfacing. All animals were given at least 24 h to recover from the surgery and get used to the experimental chamber. The entire apparatus was shielded with a one-way blind to enable observations to be made and to minimize disturbance.

In the first series of experiments ($N = 8$ animals), a blood sample was withdrawn near the end of a 2- to 3-min breathing spell. As soon as the animal voluntarily submerged, a Perspex lid was inserted between the aerial and aquatic compartments so as to prevent any further lung ventilations for 30 min. Just before the lid was removed, a second blood sample was taken (time zero). As the animal then resumed its voluntary diving–emergence behaviour, samples were taken at +1, +2, +4, +8 and +22 h following the dive. The timing of these samples was not made to coincide

with any particular part of the breathing–nonbreathing cycles. The second series of experiments ($N = 12$ animals) was designed to examine voluntary dives of similar length to the enforced 30-min submergence. When the animals were observed to exhibit their characteristic 'burst-breathing' behaviour (bursts of lung ventilations separated in time by long dives; Boutilier, 1984), blood samples were taken every 10–15 min. Owing to the unpredictable length of a voluntary dive and the fact that dives of 30 min duration are less frequent than shorter periods of submergence (Boutilier, 1984), data were collected for numerous dives of 15 min and less. Some of these data have been reported elsewhere (Boutilier, 1984).

Measurements of blood pH, P_{CO_2} and P_{O_2} were made using Radiometer (Copenhagen) electrodes and display meters (see Boutilier, 1984). Total CO_2 contents of anaerobically obtained true plasma were measured using the electrode and cuvette method of Cameron (1971). Bicarbonate concentrations ($[HCO_3^-]$) in true plasma were estimated from the measured values of total CO_2 concentration ($[CO_2]$) with the equation

$$[HCO_3^-] = [CO_2] - (\alpha CO_2 \times P_{CO_2}),$$

where αCO_2 is the carbon dioxide solubility in plasma (in mmol l^{-1} $Torr^{-1}$) at 25 °C (Reeves, 1976). The oxygen content of whole blood was determined by means of a Lex-O_2-Con apparatus (Lexington Instruments, MA). Whole blood lactate concentrations were assayed enzymatically with Sigma reagents (Sigma Kit 826-UV). Haematocrit (Hct) measurements were made by centrifugation of whole blood for 3 min at 5000 ***g*** (Gelman–Hawksley microhaematocrit centrifuge).

RESULTS

Lung ventilation following prolonged dives

Voluntarily diving *Xenopus* exhibit a wide variety of breathing patterns (cf. Boutilier, 1984), one of which ('burst breathing') is characterized by long periods of diving punctuated with comparatively short outbursts of lung ventilation. A breathing burst following a voluntary dive of 30 min or more was always essentially the same as that which immediately preceded the period of submergence (Fig. 1A,B), whereas that following a 30-min enforced dive was always markedly different (Fig. 1C,D). Analysis of the periods of breathing at the surface, both before and after a 30-min enforced dive, is shown in Table 1. The average inspiratory volume of a breath following an enforced dive was not significantly different from that seen prior to the dive. As the mean frequency of breathing was also unchanged, the inspiratory flow (minute ventilation) was the same after as before the period of enforced submergence (Table 1). The large increase in ventilation following enforced dives was therefore due to the increased amount of time spent at the surface (Table 1; Fig. 1C,D).

An analysis of the number of breaths and their associated volumes of inspired gas is shown in Fig. 2 for a single animal contributing to the mean data in Table 1. It is evident that upon emergence from an enforced dive, the frequency of breathing and

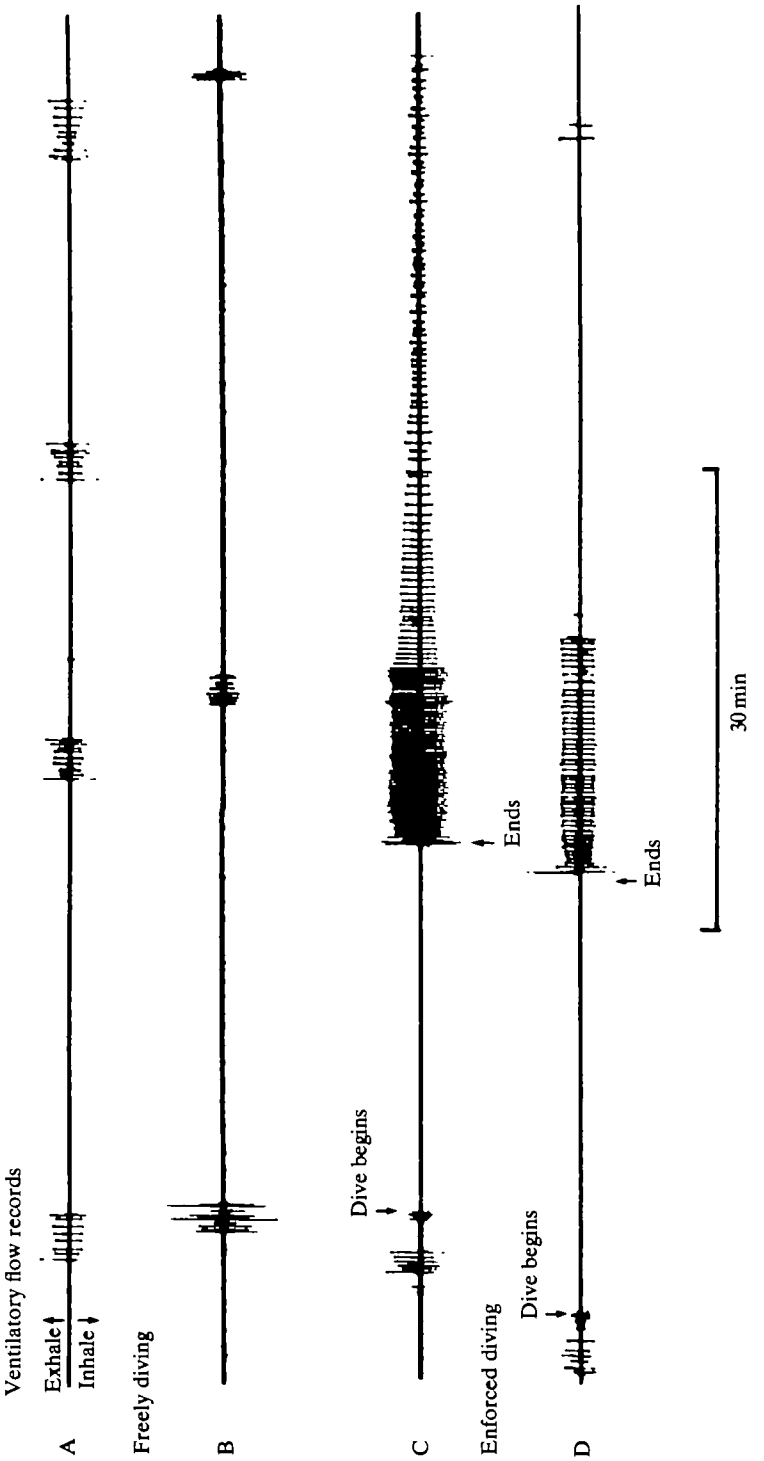

Fig. 1. Lung ventilations and periods of breath holding showing intermittent breathing pattern of voluntarily diving *Xenopus* (A,B) and the ventilation before and following two enforced dives of approximately 30 min duration (C,D). Breathing movements were detected when animals surfaced to breathe at a blowhole fitted with a pneumotachograph.

the associated inspiratory flow volumes were markedly increased; over the first 2 min of the surface period following the enforced dive, the ventilation frequency was $9{\cdot}5\,min^{-1}$ whilst inspiratory flow amounted to more than $27\,ml\,min^{-1}\,100g^{-1}$. These values are far in excess of the mean data shown in Table 1. An initial hyperpnoea following an enforced dive was more evident in some animals (Fig. 1C) than in others (Fig. 1D). As is the case for animals diving voluntarily (Boutilier, 1984), changes in the amount of gas inspired were mainly brought about by altering the amount of time spent ventilating at the surface rather than through changes in the depth of breathing (Table 1), though the latter did at times show an appreciable variation (e.g. Fig. 1C). It seems likely that the differences in breathing patterns seen after voluntary and forced dives are caused by the relatively greater anaerobic production of energy in the latter. Furthermore, the variations in the breathing patterns during recovery from enforced dives (e.g. Fig. 1C *versus* 1D) are probably the result of different levels of activity during the periods of submergence.

Blood gases and pH

The respiratory acid–base variables of arterial blood samples, taken either during or shortly after a breathing burst in a freely diving animal (levels shown at *B* in Fig. 3), fall within the range of values previously reported for this species at 25 °C (Emilio & Shelton, 1980; Boutilier, 1984). As is the case for other chronically catheterized amphibians (McDonald *et al.* 1980; Boutilier *et al.* 1980; Boutilier & Toews, 1981), the levels of arterial blood lactate were uniformly low ($0{\cdot}73 \pm 0{\cdot}10$ $mmol\,l^{-1}$). The very much higher levels of lactate ($5–10\,mmol\,l^{-1}$) and lower pH values (7·4–7·6) reported for blood obtained from *Xenopus* by cardiac puncture (Putnam, 1979; Jokumsen & Weber, 1980) are uncharacteristic of blood samples drawn from indwelling catheters in freely moving animals. Animals in the present experiments were isolated from visual and mechanical disturbances by enclosing the experimental chamber behind a blind and cushioning the entire apparatus on foam

Table 1. *Mean values (±1 S.E.M.) of ventilatory variables associated with the breathing periods before and after 30-min enforced dives in eight* Xenopus laevis

Ventilatory variables within the surface periods		
Quantity	Pre-dive	Post-dive
Burst duration (min)	$2{\cdot}7 \pm 0{\cdot}2$	$26{\cdot}8 \pm 4{\cdot}6$
Frequency of breathing during burst (breaths min^{-1})	$3{\cdot}8 \pm 0{\cdot}4$	$3{\cdot}4 \pm 0{\cdot}3$
Inspiratory volume per breath ($ml\,100\,g^{-1}$)	$4{\cdot}4 \pm 0{\cdot}5$	$4{\cdot}5 \pm 0{\cdot}9$
Inspiratory flow rate (minute ventilation) during burst ($ml\,min\,100\,g^{-1}$)	$17{\cdot}1 \pm 2{\cdot}1$	$15{\cdot}5 \pm 2{\cdot}5$
Total volume inspired in a single burst ($ml\,100\,g^{-1}$)	$44{\cdot}5 \pm 4{\cdot}4$	$413{\cdot}2 \pm 46{\cdot}6$

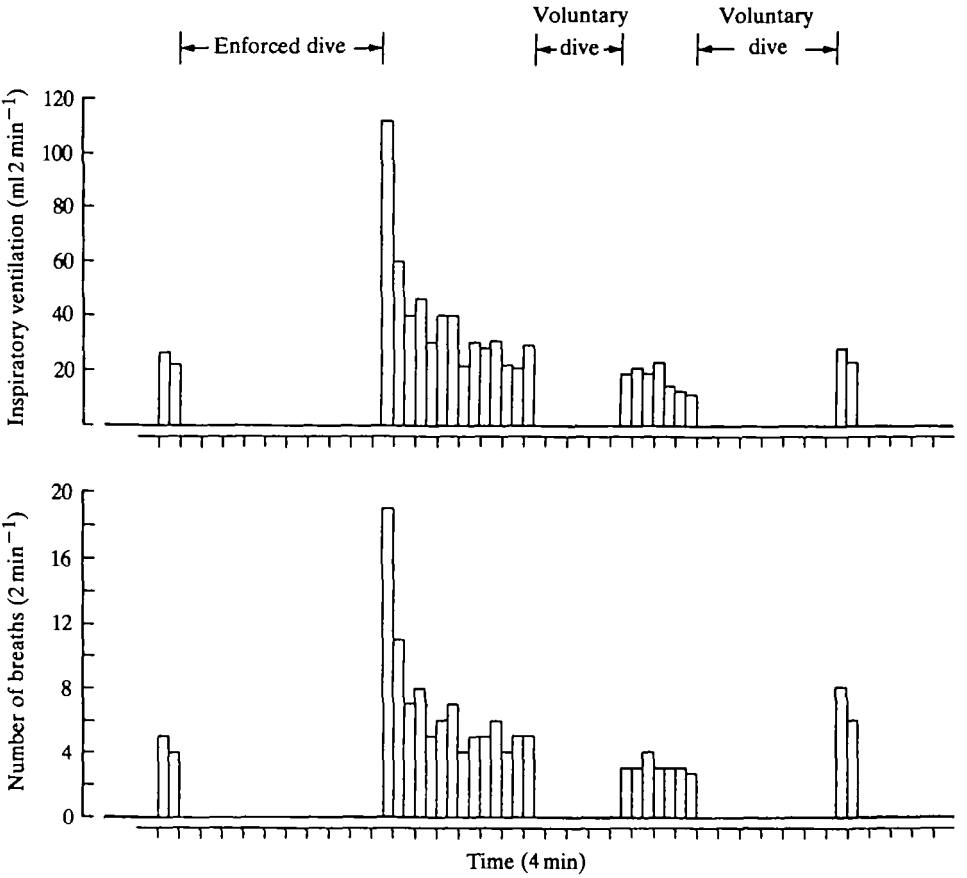

Fig. 2. The volume of inspired gas and the associated number of lung ventilations as a function of time for periods before and following a 38-min enforced dive in a 131-g female *Xenopus* at 25°C.

padding. Blood sampling under these conditions caused no apparent disturbance to the animals.

Enforced 30-min dives

Although each animal submerged voluntarily, observations after the lid was in place showed that the animals became variously engaged in active attempts to reach the surface before the 30-min time period had elapsed. In a few instances, these bouts of activity occurred early on in the 'dive' with the animal eventually returning to the bottom of the tank. Presumably, the activity associated with attempts to emerge marked what would have been the end of the voluntary period of submergence. As voluntary dives vary considerably in their length (Boutilier, 1984), it is not surprising that the onset and duration of such activity was also quite variable.

Enforced dives of this sort led to marked changes in the acid–base status of the arterial blood (Fig. 3). By the end of a 30-min dive (time 0, Fig. 3), a 10 Torr increase in arterial blood P_{CO_2} and a 4·5 mmol l^{-1} rise in blood lactate had caused the pH of the plasma to decline by 0·24 pH units. The net result of these respiratory and

metabolic acid additions to the blood was an overall reduction in the true plasma bicarbonate concentration from 29·2 to 26·4 mmol l^{-1} (time *B* to 0, Fig. 3). By 1 h following the dive, all variables had begun to change in direction, with sampling over subsequent hours revealing a gradual return to levels characteristic of the voluntarily breathing animal. Complete recovery of the acid–base status following such dives took more than 4 h (Fig. 3).

Similar patterns of change and subsequent recovery were also observed for simultaneously measured levels of arterial blood P_{O_2}, O_2 content and haematocrit (Fig. 4). During the 30-min enforced dive, arterial P_{O_2} declined from 80·8 to 20·5 Torr and O_2 content fell to 2·8 vols % from a pre-dive level of 8·5 vols %. Over the same time period, haematocrit levels increased considerably from a pre-dive mean of 26 % to a value of nearly 40 % following the dive. Between 1 and 2 h following the

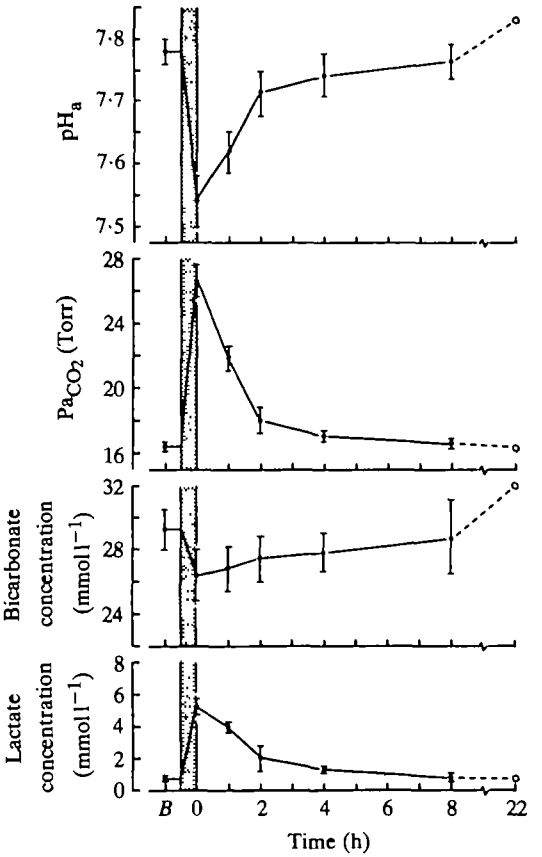

Fig. 3. Simultaneously measured pH, CO_2 tensions (P_{CO_2}) and true plasma bicarbonate and lactate concentrations in arterial blood samples from *Xenopus* before and at times after a 30-min enforced dive. *B*, pre-dive control samples taken during a breathing period; time 0, just prior to surfacing; shaded area, enforced dive period. Open circles at +22 h post-dive are mean values for three animals. All other data are means ± 1 S.E.M. for eight animals. Temperature, 25 °C.

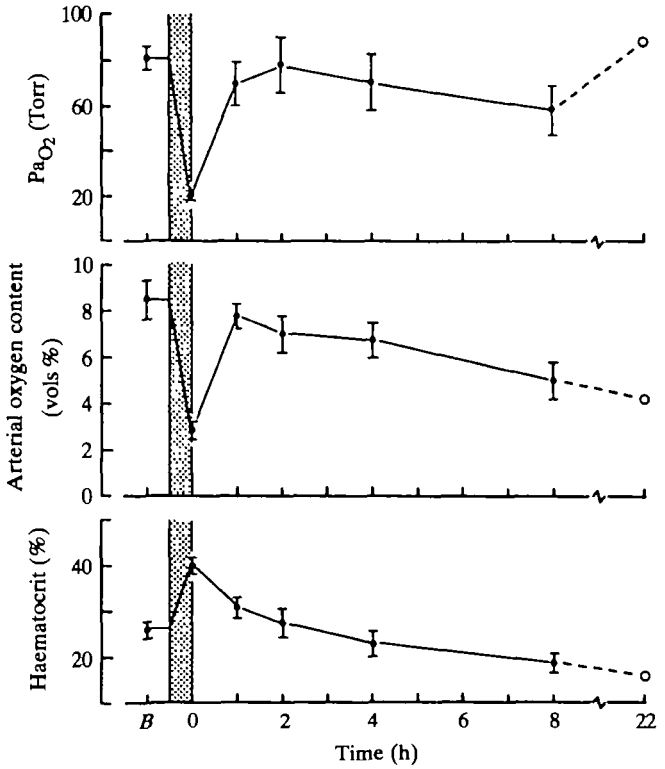

Fig. 4. Oxygen tensions (P_{O_2}), oxygen contents and haematocrit values of the same arterial blood samples as in Fig. 3.

dive, all of these variables returned to the levels seen prior to the enforced period of submergence (Fig. 4).

Prolonged voluntary dives

Initial experiments clearly showed that upon emergence from prolonged 30- to 40-min voluntary dives, the blood acid–base status and oxygenation were usually restored to normal pre-dive levels within the first breathing period (Fig. 5; Shelton & Boutilier, 1982). Arterial blood samples taken from six *Xenopus* diving freely for periods of about 30 min remained low in lactate (Fig. 6), suggesting that anaerobiosis was not important during such dives. Arterial P_{CO_2} rose and pH fell, rapidly at first, slowing later throughout the dive (Fig. 6). The acidosis was mainly respiratory in origin with little or no metabolic component. Mean data for pH, P_{CO_2} and lactate concentrations of the *Xenopus* subjected to forced dives of about the same duration as the voluntary dives also appear in Fig. 6, showing the much greater changes for forced than voluntary dives.

In both forced and voluntary 30-min dives, arterial P_{O_2} fell on average by approximately 60 Torr (Fig. 6). Voluntary dives appeared to be terminated when levels of arterial P_{O_2} reached approximately 20 Torr. Evidently, the metabolic acidosis associated with enforced dives was not the result of complete extraction of

the blood O_2 store, since the levels of arterial P_{O_2} in these animals were as high as those of the voluntarily diving animals after 30 min of submergence (Fig. 6).

DISCUSSION

There are certain problems inherent in making measurements of diving behaviour and of the changes this behaviour produces in gas exchange, cardiovascular performance and metabolic activity. Most observational and experimental methods cause the behaviour to change in some way (Butler & Jones, 1982; Butler & Woakes, 1979; Kooyman *et al.* 1980). For example, enforced dives on restrained animals, which have been used in the study of bradycardia, selective peripheral vasoconstriction and anaerobiosis of the classical diving response cause many changes that are not typical of animals making voluntary dives. Even when animals are unrestrained, the cannulation and sampling techniques, and even the presence of the experimenter, can affect diving behaviour. In *Xenopus*, for example, activity levels are higher and dives much shorter than in the totally undisturbed animal (Emilio & Shelton, 1974, 1980). In the free dives described in this paper, every precaution was taken to ensure that the animals were undisturbed, so that a valid comparison could be made with the forced dive experiments.

The evidence presented here leads us to conclude that anaerobiosis is unimportant in free dives, even when they are of considerable duration. There is no accumulation of lactic acid and the fall in blood O_2, the increase in CO_2 and the associated

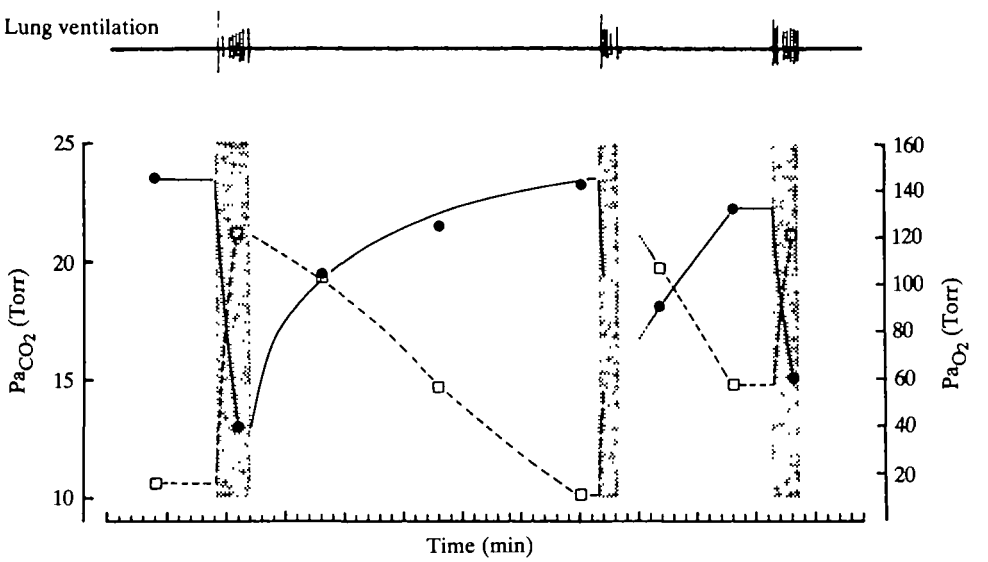

Fig. 5. Fluctuations in P_{O_2} (open squares) and P_{CO_2} (closed circles) in blood samples taken from the femoral artery of a 146-g *Xenopus* during the course of two voluntary dives at 25 °C. Lung ventilations (as recorded by a pneumotachograph) are shown in the upper trace and their timing represented by the shaded blocks on the graph.

respiratory acidosis are all corrected within the first few breaths, involving 1 or 2 min at the surface (Figs 5, 6).

In view of these conclusions, an assessment of the body's capacity to store respiratory gases, particularly O_2, and of the gas exchange through the skin, is important. Previous analyses, based on experiments on restrained animals (Jones, 1972) or on measurements of the depletion rates in lung and blood gases of active animals (Emilio & Shelton, 1974), have claimed that O_2 stores are quickly used during a dive. The O_2 requirements of undisturbed *Xenopus* at 20–25 °C, moving only to come to the surface to breathe, is 4·2 ml O_2 100 g^{-1} h^{-1} or less, of which at least 0·6 ml O_2 100 g^{-1} h^{-1} is supplied through the skin (Emilio & Shelton, 1974). In more disturbed animals, O_2 consumption is 8·6 ml O_2 100 g^{-1} h^{-1} and skin exchange is 2·5 ml O_2 100 g^{-1} h^{-1} (Emilio & Shelton, 1980). The extent of the O_2 stores may be equally variable. The degree of lung inflation can vary by several millilitres, but it seems unlikely that an animal could dive effectively with more than 7–8 ml of air in

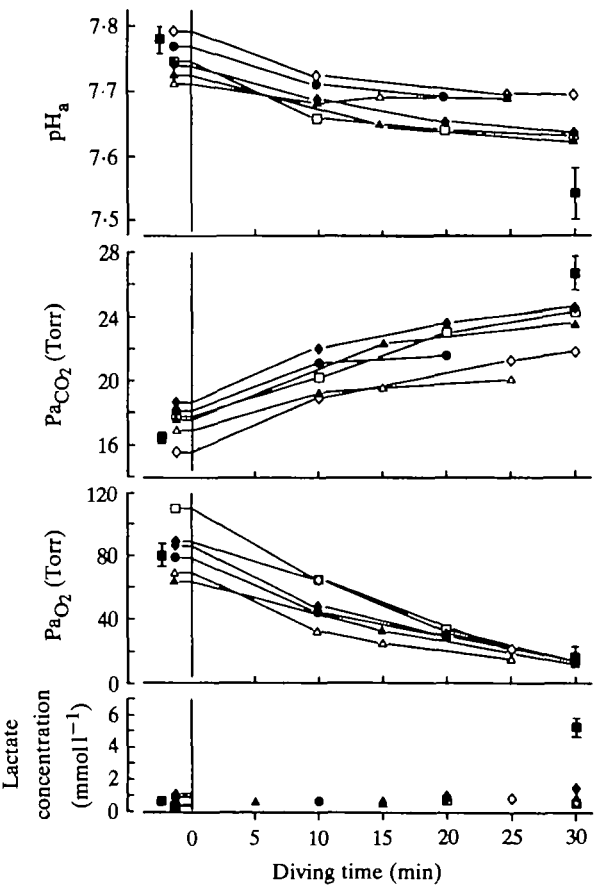

Fig. 6. Changes in pH, P_{CO_2}, P_{O_2} and lactate concentrations in blood samples taken from the femoral arteries of six *Xenopus laevis* (plotted as different symbols) during the course of prolonged voluntary dives at 25 °C. Changes produced by 30-min enforced dives (Figs 3,4) in eight animals (means ± 1 S.E.M.) are shown plotted as solid squares.

the lung. A fall in lung P_{O_2} from 120 to 30 Torr during a dive (Shelton & Boutilier, 1982) would yield 0·8 to 0·9 ml O_2. The blood volume of *Xenopus* is 13·4 ml $100\,g^{-1}$ (Emilio & Shelton, 1980) and O_2 capacity is 8·5 vols % (Boutilier & Shelton, 1986). Assuming that 25 % of the blood is arterial and fully saturated and that the remainder is venous and contains 6·3 vols %, the total blood store would be 0·9 ml O_2, of which 0·6–0·7 ml would be usable as blood P_{O_2} declined to 20 Torr. Usable tissue stores would be small, at less than 0·1 ml. The total usable store of 1·4–1·7 ml O_2 would allow an undisturbed 100-g *Xenopus* to remain submerged for 23–28 min, whereas a disturbed and more active animal would need to breathe after 14–16 min.

These estimates agree remarkably well with the present and previously reported observations (Boutilier, 1984) and substantiate the hypothesis that free dives are aerobic. Still longer aerobic dives would be possible if the estimates were rather conservative, as they probably are. There is evidence that the skin O_2 uptake increases during a dive (Emilio & Shelton, 1974). The P_{O_2} gradient across the skin increases as the dive progresses and it is also likely that capillary recruitment occurs in the skin under these conditions (Poczopko, 1957, 1959; Burggren & Moalli, 1984). More O_2 may be taken from the lung than the P_{O_2} change suggests, because lung volume declines during a dive. Finally, the estimates of tissue O_2 requirements during a dive may be too high, as they are based on averaged figures over several breathing–diving cycles. Muscle O_2 requirements must increase as the toad swims to the surface and ventilates the lungs, but these demands will be met by O_2 uptake during the breathing period itself. The tissue O_2 requirements in a negatively buoyant, completely inactive toad will therefore be less than the average figures suggest.

The accumulation and removal of CO_2 during a voluntary dive seem to present few problems. The Pa_{CO_2} increases more rapidly in the first 10 min of such a dive and causes a mild respiratory acidosis (Figs 5, 6). Towards the end of a 30-min dive, Pa_{CO_2} and pH are changing little, probably because of increased CO_2 losses through the skin. The Pa_{CO_2} values reached at the end of 30-min voluntary dives are less than those seen in forced dives because of the absence of protons of metabolic origin. In the forced dives, these products of anaerobiosis give rise to CO_2 by dehydration of bicarbonate ions.

The claim that O_2 stores are renewed and excess CO_2 removed during the first few breaths after a dive perhaps needs some qualification. Only the lung (Shelton & Boutilier, 1982) and the arterial blood components have been examined in detail. Further work on the renewal of the venous and tissue stores is in progress. However, it is known that pulmonary blood flow increases substantially when the animal is breathing and that venous blood is preferentially conveyed to the pulmocutaneous vessels in the partially divided heart (Shelton, 1976). Both characteristics are obviously of major benefit in restoring blood and tissue stores in an animal in which the lung is repeatedly ventilated during the time at the surface (Shelton, 1985).

At this stage, we can make no firm conclusions about the relevance of the changes seen in forced dives to voluntary diving behaviour. It is clear from the fall in pH and bicarbonate concentrations that metabolic protons are present in the blood of animals

after a 30-min forced dive. The accumulation of lactate is also evidence of extensive anaerobiosis, as is the prolonged recovery period (Fig. 3), which in *Xenopus* takes about 8 h to complete. However, these dives are invariably associated with increased levels of activity and the overall patterns of acid–base changes in the blood are very similar to those seen in studies of exercise, either in air or with free access to air (McDonald *et al.* 1980; Boutilier *et al.* 1980; Boutilier, Emilio & Shelton, 1986). It could be argued that O_2 stores are slowly exploited in inactive dives but that sudden bursts of mechanical power and of energy consumption in the voluntary musculature are served very largely by anaerobic pathways, even though the stores may still contain some O_2. *Xenopus*, in common with many other amphibians and reptiles (Shelton & Boutilier, 1982), can survive prolonged submergence with marked acidoses and lactate accumulations, well beyond the limits of the 30-min dives described in this paper. The ability to tolerate high concentrations of anaerobic end-products may be an adaptation to high activity levels (Bennett & Licht, 1973, 1974; Hutchison & Miller, 1979*a,b*), prolonged diving, or both. Unfortunately the present experiments do not show whether free dives are ever extended to the point where available O_2 stores are completely depleted and anaerobiosis begins.

The range of both internal and external conditions under which extended dives may begin and end are largely unknown. They are worth investigation not only to test whether anaerobiosis can be an important component in a prolonged dive but also to give more insight into the complexities of breathing and diving behaviour. The present experiments suggest that prolonged voluntary dives end and breathing begins when O_2 stores are reduced to certain levels. The relative constancy of CO_2 and pH indicates that these factors are of lesser importance. Yet, they cannot be totally ignored because breathing is stimulated to begin at relatively high levels of Pa_{O_2} if Pa_{CO_2} is also high (Shelton & Boutilier, 1982). Similarly, it could be argued that a breathing period ends when the O_2 store has been renewed. However, lung ventilations continue for some time after alveolar and arterial P_{O_2} are restored to high levels (Shelton & Boutilier, 1982) and so it is also necessary to suppose that the tissue and venous compartments of the store are monitored (Shelton, 1985). In addition other factors, such as the acid–base balance in blood and tissues, must be of importance in ending a breathing period because ventilation at the end of a forced dive seems to be too prolonged simply to be involved in renewing the O_2 stores. Until the full repertoire of these intermittently breathing animals has been studied, the precise nature of the control processes will continue to be elusive.

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