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RESPIRATORY PROPERTIES OF BLOOD FROM VOLUNTARILY AND FORCIBLY SUBMERGED *XENOPUS LAEVIS*

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SUMMARY

The respiratory properties of blood from voluntarily diving *Xenopus* seem well matched to the animal's habit of ventilating its lungs in an intermittent fashion. Compared with more terrestrial anurans, the high oxyhaemoglobin affinity (P_{50} = 29·6 Torr, pH 7·73, 25 °C), Bohr effect ($\Delta\log P_{50}/\Delta pH = -0{\cdot}37$) and Haldane effect (0·37 mol CO_2 mol^{-1} O_2) can be viewed collectively as adaptations towards effective blood gas storage during periods of apnoea and blood gas exchange during episodes of air breathing. It appears, therefore, that these biochemical adaptations are linked to the changes in respiratory blood flow that are made possible by the partially divided double circulation of *Xenopus*.

In comparison with blood from voluntarily diving *Xenopus*, that taken from animals at the end of a 30-min enforced dive was haemoconcentrated and contained 4 and 8 mmol l^{-1} higher concentrations of true plasma lactate and metabolic acid equivalents respectively. The pH-induced effects of the latter led to reductions in oxyhaemoglobin affinity and blood CO_2 carriage, both of which persisted for up to 4 h following emergence from an enforced dive. The associated haemoconcentration led to a secondary series of effects of which the most obvious were an elevated blood oxygen-carrying capacity and an increased non-bicarbonate buffer slope for true plasma. Marked changes, such as these, were never observed in blood samples taken at various stages of voluntary dives lasting upwards of 30 min.

INTRODUCTION

Xenopus laevis is an aquatic anuran which relies on periodic episodes of air breathing for the major portion of its oxygen uptake. This behaviour causes substantial oscillations in respiratory gases in lungs, blood and tissues (Shelton & Boutilier, 1982). It has been argued that oscillations of this type are an inevitable consequence of the evolution of air-breathing in fishes and early tetrapods (Shelton, Jones & Milsom, 1985). It also seems likely that the early evolution of a partially

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divided, double circulation is linked to the use of the blood as a store for respiratory gases (Shelton, 1985). If these views are to be sustained, it is important to understand the effects of intermittent ventilation on all parts of the gas exchange and storage systems.

In *Xenopus*, short (10 min) voluntary dives cause blood O_2 partial pressures to fall from approximately 80 to 40 Torr (Emilio & Shelton, 1974). At times, however, the animal will remain voluntarily submerged for periods of up to 1 h (Boutilier, 1984), though the corresponding details of blood gases are not known. In a recent study in which *Xenopus* was allowed to dive voluntarily but then restricted from reaching the surface, Emilio & Shelton (1980) found that a mild respiratory acidosis developed over the first 10 min. Longer dives of this sort, up to 30 min duration, caused a combined respiratory and metabolic acidosis, the latter persisting for some hours after the animal surfaced and breathed. Associated with this acidosis was a considerable haemoconcentration resulting from a loss of plasma water to some extravascular compartment. Similar observations have been made on other forcibly submerged amphibians (Jones, 1972; Jones & Mustafa, 1973; Emilio, 1974; Lillo, 1978). These and other studies have led to a view that anaerobic pathways are an integral part of these animals' normal diving metabolism. However, there is an increasing amount of evidence to show that periods of voluntary submergence end before O_2 stores are fully depleted (Lenfant & Johansen, 1967; Toews, 1971; Toews, Shelton & Randall, 1971; Emilio & Shelton, 1974, 1980). In the present study, we examine the effects of forced and voluntary dives on the oxygen-carrying and acid–base properties of *Xenopus* blood in order to understand the changes that can be produced in this important gas transport and storage medium as the animal breathes intermittently.

MATERIALS AND METHODS

Preparation of experimental animals

Male and female specimens of the South African clawed toad, *Xenopus laevis*, were either obtained commercially or selected from a laboratory reared stock. All animals were kept in large communal tanks (20–25 °C) and fed on chopped liver twice weekly. At least 1 week prior to experimental procedures, the animals were transferred to laboratory tanks (25 ± 2 °C) where feeding was suspended.

After anaesthesia, induced by immersion in a 0·06 % solution of tricaine methanesulphonate (MS-222, Sandoz; titrated to pH 7·0 with NaOH), a catheter was chronically implanted in the femoral artery of each animal (Boutilier, 1984). The toads were then artificially ventilated until they had recovered and they were given at least 24 h in which to acclimate to the experimental chambers (25 ± 0·5 °C). The P_{O_2} of the water was >140 Torr at all stages of the experiments, $P_{CO_2} < 0{\cdot}5$ Torr (Radiometer electrodes and meters).

Oxygen and carbon dioxide properties of blood and plasma

Acid–base and oxygenation characteristics of whole blood and plasma were studied by *in vitro* tonometry at 25 °C. Individual blood samples were drawn from

the femoral cannulae of several animals, pooled to the required volumes, and stored at 4°C for less than 1 h before use. Aliquots having the same haematocrit as the parent blood pool were transferred to tonometers (50 ml round bottom glass flasks) and equilibrated with a wide range of gas mixtures produced by Wösthoff pumps (Bochum, FRG). Blood samples for analyses were drawn after a predetermined 30-min equilibration time, into positive displacement gas-tight Hamilton syringes.

Whole blood oxygen equilibrium curves were constructed at several P_{CO_2} (and thus pH) levels using the mixing method (Haab, Piiper & Rahn, 1960) as detailed further by Scheid & Meyer (1978). Blood samples were equilibrated against gas mixtures containing various concentrations of CO_2 in air and CO_2 in nitrogen. Samples of known O_2 saturation were analysed for P_{O_2}, P_{CO_2}, pH and haematocrit, with replicate samples from the 2% CO_2/air-equilibrated tonometer being used for measures of total blood O_2-carrying capacity (i.e. at approximately 14·5 Torr P_{CO_2} and 155 Torr P_{O_2}). The oxygen content of separated plasma was determined also on samples equilibrated with gas mixtures containing 2% CO_2-balance air. The relationship between blood O_2-carrying capacity and haematocrit was assessed over a wide range on blood whose haematocrit was experimentally altered to high and low levels by centrifugation and resuspension at various red blood cell to plasma volume ratios.

In vitro acid–base relationships were determined using the same apparatus as above. Measurements of pH, P_{CO_2}, lactate concentrations, total CO_2 concentrations and haematocrit were made on blood equilibrated against humidified gas mixtures containing various proportions of CO_2 in either air or nitrogen. The true plasma of each blood sample, obtained by centrifugation of blood in a sealed capillary tube, was analysed for total CO_2 concentration. To allow comparison, the above procedures were carried out on blood obtained from unrestrained, freely-diving animals (67–101 g, $N = 31$) and from animals at the 30 min mark of an enforced dive (62–93 g, $N = 19$).

Blood respiratory properties before, during and following enforced dives

Twelve toads (80–120 g) were prepared with arterial catheters and allowed to recover as before. Blood samples were drawn initially from each animal as it was freely diving and surfacing in air-saturated water. By lowering a Perspex lid over the water surface, the animal was forced to remain submerged for 30 min at which time a second blood sample was taken. The lid was then removed from the surface and additional blood samples (400 μl) were drawn at +1, +2 and +4 h following the 'dive'. The determinations were carried out on groups of three animals; the blood samples (400 μl animal^{-1} stage^{-1}) were pooled, stored on ice, and within 1 h the blood pool was divided equally between an oxygenated and deoxygenated tonometer. Following equilibration with 2% CO_2/air or 2% CO_2/N_2 gas mixtures, known blood O_2 saturations were prepared by the mixing method (approximately 10, 50 and 90% saturations). Measurements made on each of these prepared samples included P_{O_2}, pH, haematocrit and true plasma concentrations of both total CO_2 and lactate.

Analytical procedures

Methodologies for the measurement of haematocrit, pH, P_{CO_2}, total CO_2, PO_2 and lactate have been detailed previously (Boutilier, Randall, Shelton & Toews, 1977; Boutilier, McDonald & Toews, 1980; Boutilier & Toews, 1981; Boutilier, 1984). Oxygen contents were determined with a Lex-O_2-Con analyser (Lexington Instruments, MA) using, for whole blood, a sample volume of 20 μl. For low O_2 contents (i.e. plasma), a calibration curve between 0 and 2 vols % was constructed by injecting known volumes of O_2/N_2 equilibrated distilled water.

Bicarbonate concentrations ($[HCO_3^-]$) in true plasma were estimated from our 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 plasma carbon dioxide solubility (in mmol l^{-1} $Torr^{-1}$) at 25 °C (Reeves, 1976). The proportion of the total blood oxygen-carrying capacity which could be attributed to oxyhaemoglobin binding was obtained by subtracting the dissolved fraction of O_2 in plasma from the blood O_2 capacity (note that all O_2 capacity measurements were made at approximately 14·5 Torr P_{CO_2} and 155 Torr P_{O_2}).

RESULTS

The effective increase in haemoglobin concentration of blood from forcibly submerged *Xenopus laevis* was reflected *in vitro* by large differences in oxygen and carbon dioxide combining properties when compared with blood samples from unrestrained animals (Table 1).

Table 1. In vitro *oxygenation and acid–base characteristics of blood samples obtained from voluntarily diving* Xenopus *and for those drawn at the end of a 30-min enforced dive*

	Voluntary	Enforced
Haematocrit (%)*	28·3 ± 0·7 (25)	46·2 ± 2·8 (21)
True plasma buffer slope ($\Delta[HCO_3^-]/\Delta pH$)	−15·0 ± 2·1 (7)	−24·6 ± 3·0 (7)
Oxyhaemoglobin carrying capacity (vols %)*	9·38 ± 0·6 (25)	15·4 ± 2·1 (21)
Plasma O_2 carrying capacity (vols %)*	0·58 ± 0·03 (8)	—
P_{50} (Torr)†	29·6 ± 1·3 (10)	37·0 ± 1·9 (5)
CO_2 Bohr effect ($\Delta \log P_{50}/\Delta pH$)‡	−0·37 ± 0·03 (12)	−0·29 ± 0·02 (7)
Lactate concentration (mmol l^{-1})	0·95 ± 0·16 (11)	5·29 ± 0·54 (9)

Values are means ± 1 S.E.M. (no. of determinations on independent blood pools).
Temperature = 25 °C.
* At 2 % CO_2-balance air equilibration gas.
† At ≃14·5 Torr P_{CO_2}.
‡ At 50 % blood O_2 saturation.

Blood oxygen characteristics in vitro

Oxygen equilibrium curves for whole blood samples drawn from animals engaged in voluntary diving-emergence behaviour and for those following a 30-min enforced dive are illustrated in Fig. 1. Individual curves contributing to each of the averaged curves shown were determined at six O_2 saturation levels (approximately 10, 20, 50, 60, 70 and 90 %). For any given level of P_{CO_2}, the P_{50} value of blood from forcibly submerged *Xenopus* was greater than that determined for the freely diving animal, as were the concentrations of lactate and free hydrogen ions (Table 1; Fig. 1). The large haematocrit increase following a forced dive and the corresponding increase in blood O_2-carrying capacity, meant that this blood *in vitro* carried greater amounts of chemically bound oxygen for any given level of P_{O_2}.

The CO_2 Bohr effect (Fig. 2) was determined by linear regression analysis of all data points obtained at 50 % saturation from the oxygen equilibrium curves of both voluntarily and forcibly submerged animals (Fig. 1). Though the slope of the $\Delta\log P_{50}/\Delta pH$ relationship was evidently reduced in blood taken from involuntarily diving toads (Fig. 2), statistical comparisons of the slopes are not offered as they represent data collected over two different pH ranges.

The relationship between whole blood O_2-carrying capacity (C_bO_2) and haematocrit (Fig. 3) was described by the equation; $C_bO_2 = 0{\cdot}339$ (haematocrit)$+0{\cdot}61$ (correlation coefficient, $r = 0{\cdot}985$, $N = 45$). As expected, the y-intercept of this equation is virtually identical to the measurement of the O_2-carrying capacity of plasma (Table 1; Fig. 3). Data in Fig. 3 are values for freely diving and forcibly submerged animals with additional points at high and low haematocrit values being obtained by experimentally preparing various red blood cell to plasma volume ratios. Note that the points for each group of animals are clustered on discrete portions of the line, the higher values being attributable to the haemoconcentration which occurred during a 30-min enforced dive (Table 1).

In vitro *CO_2 relationships*

An increase in P_{CO_2} causes the red blood cells to swell in *Xenopus* as in mammals. This swelling is reflected as an increased haematocrit as Fig. 4 shows. Determinations of oxyhaemoglobin-carrying capacity made at the same time as the haematocrit showed that it did not change with equilibration gas P_{CO_2}, confirming that the haematocrit variations were due to cell swelling. Plasma trapping during centrifugation can amount to 2–3 % in packed red cells of *Xenopus* (Emilio & Shelton, 1980) and, though this may change as a result of increased red cell size, the effects will be much smaller than those measured here.

Xenopus whole blood exhibited a Haldane effect: at any given P_{CO_2} level, the whole blood total CO_2 content and pH were significantly lower in oxygenated than deoxygenated blood (Fig. 5). The mean difference in total CO_2 content between oxygenated and deoxygenated blood was found to be $1{\cdot}4\ mmol\ l^{-1}$ (0·045–0·070 pH units difference). Accordingly, the Haldane effect is expressed as the ratio of the change in blood total CO_2 content between oxygenated and deoxygenated blood over

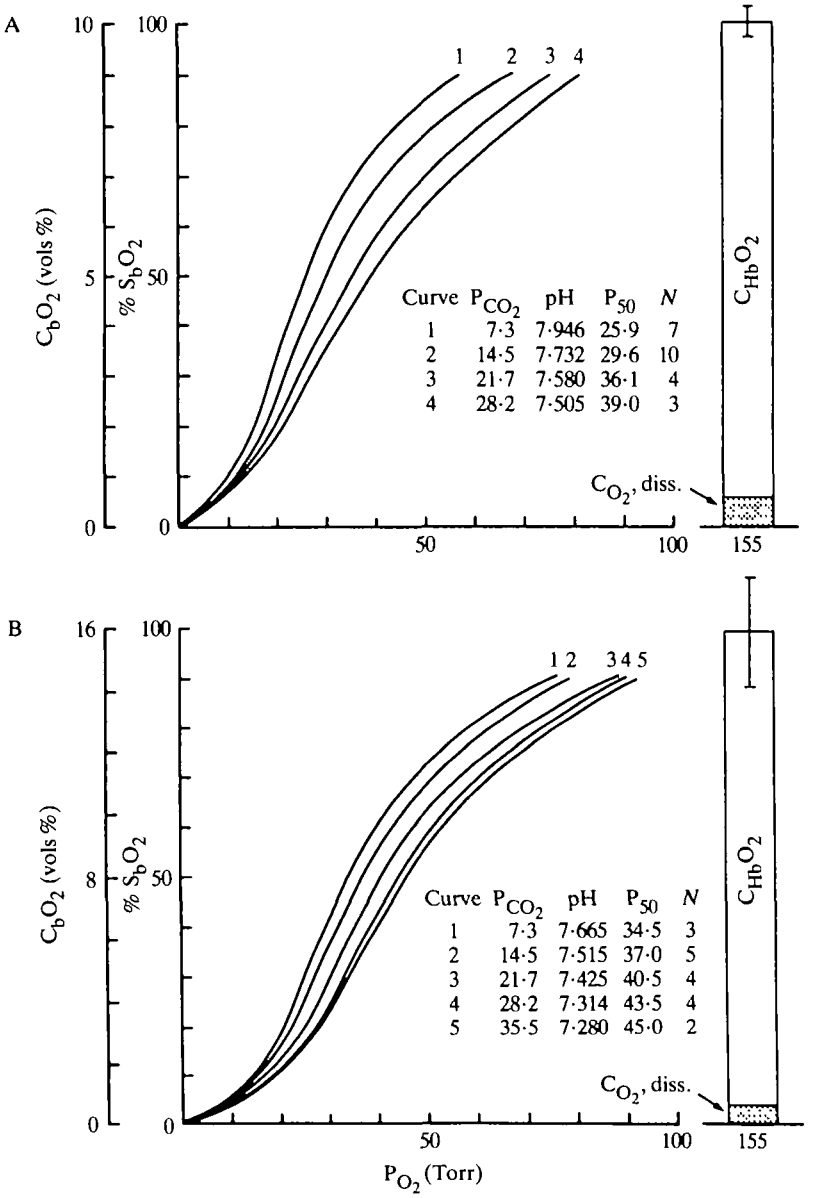

Fig. 1. *In vitro* oxygen equilibrium curves of whole blood taken from (A) freely diving and (B) forcibly submerged *Xenopus* at 25°C. Curve numbers are percentages of equilibration gas CO_2. Tabulated data are mean values for blood acid–base status at the half saturation value P_{50}. *N*, number of independent curve determinations on separate blood pools; C_bO_2, whole blood oxygen-carrying capacity at ≃155 Torr P_{O_2}; S_bO_2, whole blood O_2 saturation; C_{HbO_2}, oxyhaemoglobin carrying capacity at ≃155 Torr P_{O_2}; C_{O_2}, diss., plasma O_2 capacity at ≃155 Torr P_{O_2}. Histogram bars show the mean blood O_2-carrying capacity (±1 S.E.M.) partitioned into oxyhaemoglobin carrying capacity and plasma O_2 capacity.

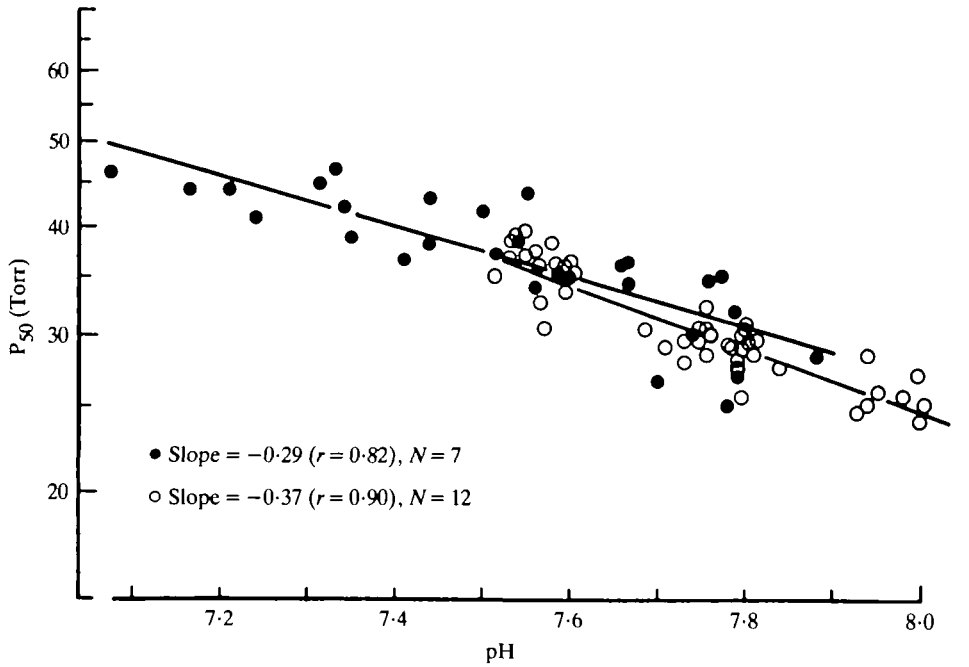

Fig. 2. *In vitro* CO_2 Bohr effect of whole blood obtained from freely diving (open circles) and forcibly submerged (closed circles) *Xenopus* at 25°C. Slopes and correlation coefficient, r, determined by least squares regression of the relationship $\Delta\log P_{50}/\Delta pH$. N, number of independent experiments on different blood pools.

the corresponding change in blood O_2 content. In Fig. 5, the change in blood O_2 content is equal to the O_2-carrying capacity of the oxygenated blood (8·5 vols% or 3·8 mmol $O_2 l^{-1}$ of blood). The ratio (i.e. Haldane effect) amounts, therefore, to 0·37 mol CO_2 mol^{-1} O_2.

Compared with voluntarily diving animals, the non-bicarbonate buffering capacity of true plasma from forcibly submerged animals increased, due to the haemoconcentration effect (Table 1). Analyses of the individual contributing slopes showed that the true plasma buffering capacity ($\Delta[HCO_3^-]/\Delta pH$) is linearly related to the blood O_2-carrying capacity (C_bO_2) by the equation $\Delta[HCO_3^-]/\Delta pH = 1{\cdot}46C_bO_2 + 1{\cdot}02$ ($r = 0{\cdot}90$).

Blood respiratory properties before, during and following enforced dives

The data in Fig. 6 summarize the time course and nature of the changes in blood characteristics following an enforced 30-min dive. Hydrogen ions from metabolic sources were clearly present after a dive because pH fell at a constant P_{CO_2} of 14·5 Torr. True plasma bicarbonate concentrations of blood tonometered at 14·5 Torr P_{CO_2} also declined during the enforced dive (Fig. 6). Using a HCO_3^-–pH diagram and the relationship between haematocrit and plasma buffering capacity, each of these post-dive bicarbonate concentrations was backtitrated along its respective buffer slope to the level which would be present at the pre-dive control pH (cf. Wood, McMahon & McDonald, 1977; McDonald, Boutilier & Toews, 1980).

The difference in bicarbonate concentration between the control pH and that after backtitration is a measure of the concentration change in metabolically originating hydrogen ions (Woodbury, 1974). Fig. 7 is a plot of the changes in true plasma concentrations of metabolic acid (in mmol l^{-1}) and lactate following enforced dives of the animals in Fig. 6. These measurements were made on blood samples prepared at 10 % O_2 saturation (for time zero) and 90 % O_2 saturation (for times B, +1, +2 and +4 h) which are realistic estimates of the state of oxygenation of the blood at these times in the experimental cycle. The Haldane effect of blood O_2 saturation on CO_2 carriage (Fig. 5) is thus taken into account in the plot of metabolic acid equivalents in Fig. 7. Changes in true plasma concentrations of metabolic acid and lactate were similar in direction but quantitatively quite different over the first 2 h following an enforced dive (Fig. 7). During that time, the lactate concentration was approximately 50 % that of the metabolic acid, both subsequently declining to pre-dive levels by the fourth hour of recovery.

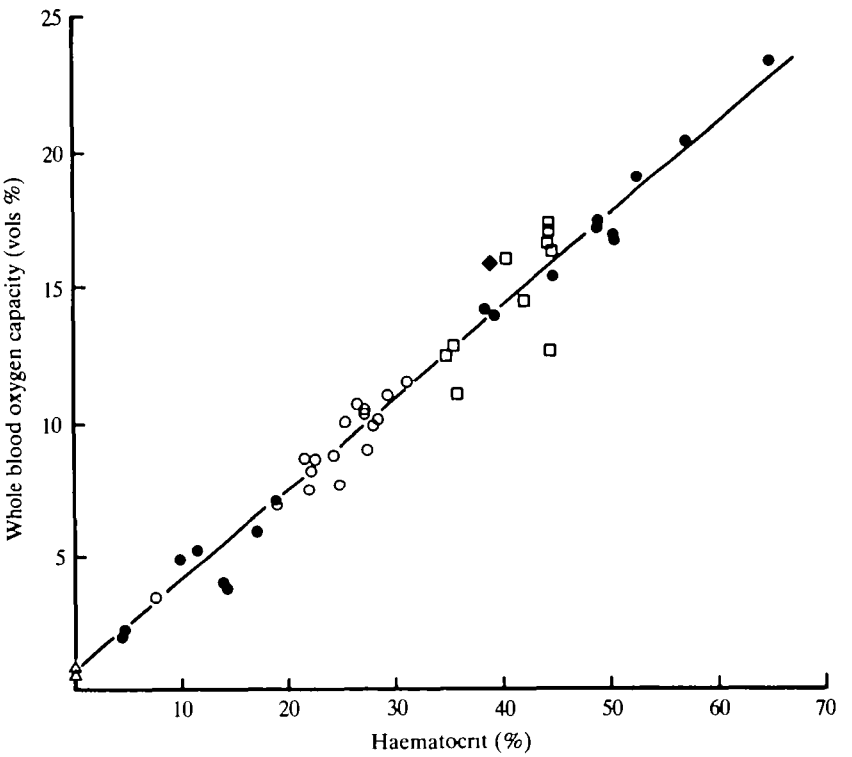

Fig. 3. Relationship *in vitro* between whole blood O_2-carrying capacity and haematocrit of 2 % CO_2/98 % air-equilibrated whole blood and separated plasma. Data shown for blood obtained from freely diving (○) and forcibly submerged (□) *Xenopus* at 25 °C. 'Haematocrit preparations' (●) represent blood whose haematocrit was experimentally altered to high and low levels by centrifugation and resuspension at various red blood cell to plasma volume ratios. Values at zero haematocrit are measurements of the O_2-carrying capacity of separated plasma (△) determined at ≃155 Torr P_{O_2}. See text for regression analysis.

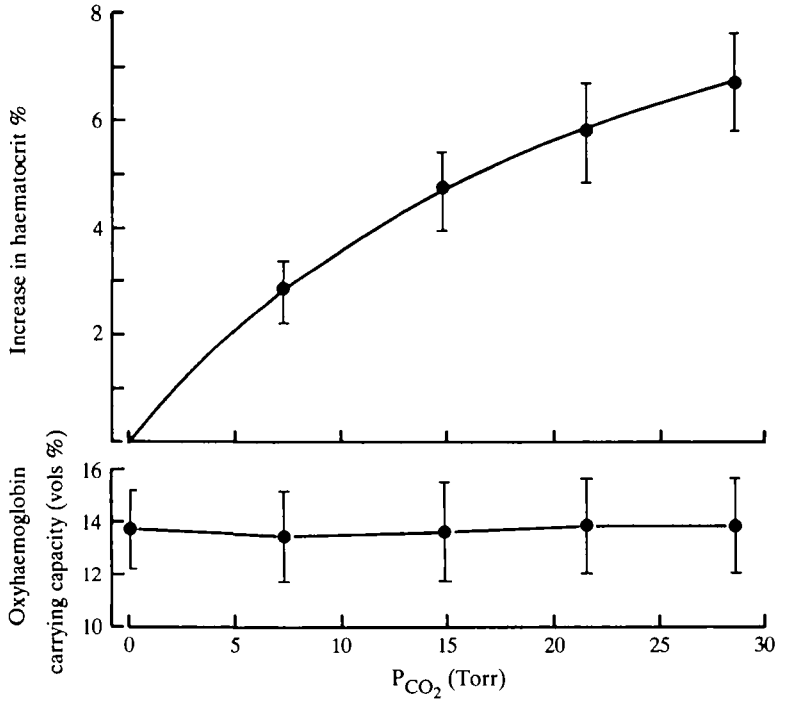

Fig. 4. Effects of step changes in equilibration gas P_{CO_2} on haematocrit of tonometered blood of *Xenopus* at 25 °C ($N = 5$ experiments). Haematocrit data (±1 S.E.M.) are plotted as the increase in haematocrit percentage (i.e. the difference between the haematocrit at any given level of P_{CO_2} and that of the same blood sample tonometered against air); line fitted by inspection. Lower panel shows mean (±1 S.E.M.) oxyhaemoglobin carrying capacity of same blood tonometered against each of the CO_2-balanced air gas mixtures.

DISCUSSION

The blood O_2-carrying capacity of voluntarily diving *Xenopus* is similar to that observed in other amphibians (Lenfant & Johansen, 1967; Wood, Weber, Maloiy & Johansen, 1975). The comparatively higher O_2-carrying capacity for *Xenopus* blood, as reported by Jokumsen & Weber (1980), is probably explicable in terms of acute blood sampling methods since both the blood pH and haematocrit were evidently affected in the same fashion as the forcibly submerged animals of the present study. Oxyhaemoglobin affinities (at 25 °C) of the semi-terrestrial anurans *Rana catesbeiana* ($P_{50} = 42$ Torr at pH 7·79), *Rana brevipoda* ($P_{50} = 52$ Torr at pH 7·72; Tazawa, Mochizuki & Piiper, 1979) and *Bufo marinus* ($P_{50} = 53$ Torr at pH 7·75; Boutilier & Toews, 1981) are very much lower than those found at comparable temperatures and pH levels in blood from voluntarily diving *Xenopus* (Table 1; Fig. 1). The P_{50} value of 29·6 Torr at pH 7·732 (Fig. 1) is similar, however, to that found in the aquatic urodele, *Amphiuma tridactylum* ($P_{50} = 27$ Torr at 22 °C; Lenfant & Johansen, 1967), an animal which, like *Xenopus*, punctuates long periods of diving with comparatively short outbursts of ventilation (Toews *et al.* 1971). Furthermore, the Bohr coefficient of blood from *Xenopus* (−0·37; Table 1; Fig. 2) is much higher than those seen in the ranids and *Bufo* (−0·18 to −0·27; Tazawa

et al. 1979; Boutilier & Toews, 1981). The Haldane coefficient in *Xenopus* blood (0·37) is identical to that of the Bohr effect, this being the prediction of the linkage equation (Wyman, 1964; Heck, 1970). Though comparable data on other anurans are not available, the linkage between coefficients suggests that a higher Bohr effect in *Xenopus*, relative to other more terrestrial anurans, will mean that the Haldane effect in *Xenopus* is also higher than in the other species. We could find no evidence for a Root effect in *Xenopus* whole blood, despite its apparent presence in haemoglobin solutions (Perutz & Brunori, 1982). All other interrelationships between haematocrit and O_2 and CO_2 carriage resemble conventional mammalian patterns.

The *in vitro* O_2 and CO_2 combining properties of blood samples taken during periods of lung ventilation were identical with those taken at various stages of voluntary dives lasting 10–30 min. In contrast, enforced 30-min dives led to a large haemoconcentration and blood acidosis (Table 1; Fig. 6). The rise in blood lactate concentrations in the forcibly submerged animal (Fig. 7) indicated that the diving O_2

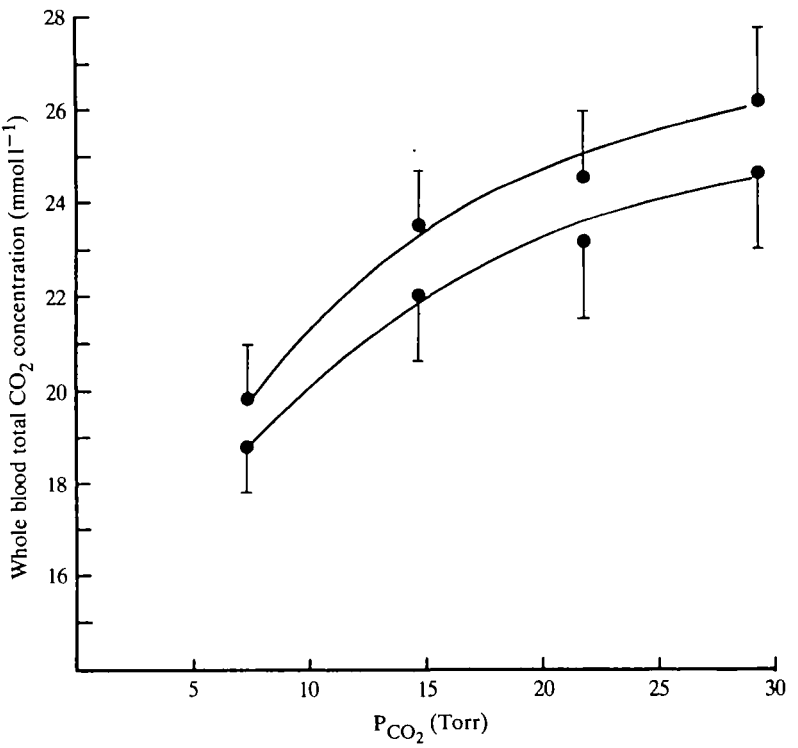

Fig. 5. *In vitro* relationship between the total CO_2 concentration of *Xenopus* whole blood at 25 °C as a function of the partial pressure of CO_2 (P_{CO_2}). The upper curve corresponds to completely deoxygenated blood, the lower curve to blood equilibrated at ≈155 Torr P_{O_2}. At any given level of P_{CO_2} the mean total CO_2 concentrations ($N = 5$ independent experiments on separate blood pools) between oxygenated and deoxygenated blood are significantly different (Student's paired t-test, $P < 0{\cdot}05$). Curved lines fitted by inspection. Values shown are means (±1 S.E.M.). Variations about means are due to differences in total CO_2 concentration between blood pools and not to variation between oxygenated and deoxygenated condition.

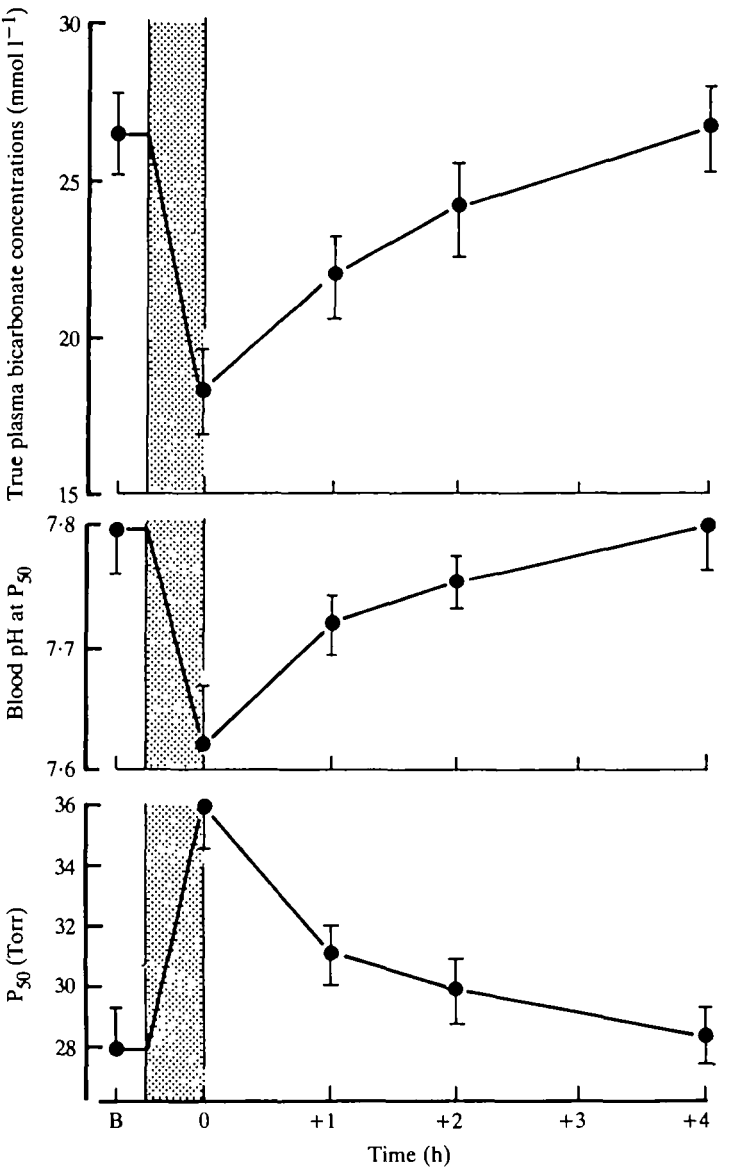

Fig. 6. pH, P_{50} and true plasma bicarbonate concentrations of blood samples taken before (B), immediately following (time 0) and +1, +2 and +4 h after an enforced 30-min dive (shaded block). Blood at each stage was a pooled sample from three animals run in parallel. Four such experiments contribute to the mean (±1 S.E.M.) values shown. Blood pools at each stage were divided between oxygenated and deoxygenated tonometers whose equilibration gas mixtures were held constant at 2% CO_2 (≃14·5 Torr P_{CO_2}). pH values shown correspond to P_{50} samples prepared by the mixing method. True plasma bicarbonate concentrations were determined on blood samples prepared at P_{90} for times B, +1, +2 and +4 at P_{10} for time 0 (see text for further explanation).

store was not adequate to supply the full energetic demands of the dive and that a switch to anaerobiosis had occurred.

Significant differences between the true plasma concentration changes in metabolic acid and lactate following an enforced dive (Fig. 7) are reminiscent of the post-activity changes seen in the blood of other species of amphibians (McDonald *et al.* 1980; Boutilier *et al.* 1980) and some fishes (Wood *et al.* 1977; Turner, Wood & Hōbe, 1983). In all instances, the concentration of metabolic acid equivalents was significantly higher than lactate for some hours following the enforced behaviour. The most obvious resolution of this discrepancy would be if hydrogen ion production occurred *via* metabolic pathways whose end-products were other than lactate, though evidence for this is scarce (cf. Bennett, 1978). The generation of protons by metabolic processes and their degree of stoichiometry with fermentable substrates is complex and controversial (Gevers, 1977; Hochachka & Mommsen, 1983; Pörtner, Heisler & Grieshaber, 1984). The most favoured possibility for the discrepancies observed in this and other studies is that protons and lactate anions are released to and/or removed from the blood at different rates.

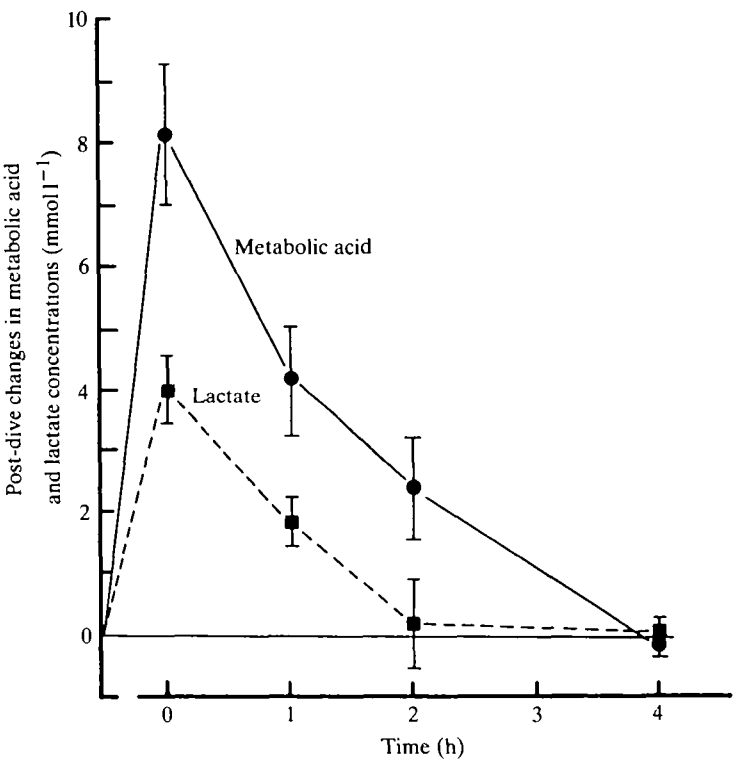

Fig. 7. Changes in true plasma concentrations of metabolic acid and lactate of the animals in Fig. 5. Note that metabolic acid (in mmol l^{-1}) is measured as the change in true plasma bicarbonate concentration at constant P_{CO_2} plus the portion of the metabolic acid concentration which is buffered by non-bicarbonate buffers according to the *in vitro* plasma buffer slope (see Fig. 6 and text).

Experiments on working gastrocnemius muscles of *Xenopus* have shown *in vivo* and *in vitro* that the haemoconcentration of an enforced dive may be explained in part by plasma water uptake into the hypoxic cells of lactate-enriched muscle tissue (Boutilier, Emilio & Shelton, 1986). Presumably, a small component of the raised haematocrit *in vivo* results from CO_2-induced red cell swelling (Fig. 4), since arterial P_{CO_2} levels are elevated by approximately 10 Torr during a 30-min enforced dive (Emilio & Shelton, 1980). There is much recent evidence that red blood cell swelling also occurs as a result of hormone-sensitive ion transport systems in erythrocytic membranes and that the associated changes in pHi, Donnan ratio, and dilution of organic polyanions can influence the respiratory properties of the blood (Palfrey & Greengard, 1981; Weber, 1982; Nikinmaa, 1983; Baroin, Garcia-Romeu, Lamare & Motais, 1984). Certainly, red cell swelling cannot account for the changes in haematocrit which lead to an elevated O_2-carrying capacity of the blood and an increased non-bicarbonate buffering ability. Thus, the rise in haematocrit observed here must be largely due to haemoconcentration, increasing the buffer slope during an enforced dive (Table 1; Fig. 6) and reducing the change in blood pH that would otherwise occur. In addition, the delivery of oxygen to hypoxic tissues will be facilitated by the decreased oxyhaemoglobin affinity (Fig. 1).

Amphibians are noted for their ability to tolerate wide internal fluctuations in body oxygenation, acid–base balance and anaerobic end-product concentrations. In diving forms, these capabilities have often been viewed as metabolic adaptations that allow diving times to be greatly extended as a part of the animals' natural behaviour. Though a shift to anaerobic pathways may at times become necessary as a short-term solution to increased energy demands, this is probably avoided during most voluntary diving conditions (Shelton & Boutilier, 1982; Boutilier, 1984).

The respiratory properties of blood taken from freely moving animals seem well matched to their predominantly aquatic existence and periodic nature of air breathing. For example, the high oxyhaemoglobin affinity and Bohr and Haldane effects (Figs 1, 2, 5), relative to more terrestrial anurans (cf. Wood *et al.* 1975; Boutilier & Toews, 1981), can be viewed as adaptations aimed at reducing the amount of time required for effective blood gas turnover whilst ventilating at the surface. In *Xenopus*, these biochemical adaptations appear in unison with a cardiovascular arrangement which permits high blood flow to the lung during breathing (Shelton, 1970, 1976; Emilio & Shelton, 1972; Shelton & Boutilier, 1982). This is consistent with the observation that all movements of the buccal pump in *Xenopus* are concerned with lung filling and emptying whereas much of the ventilatory pattern of ranid and bufonid species involves non-pulmonary buccal oscillations (DeJongh & Gans, 1969; Macintyre & Toews, 1976; West & Jones, 1975); *Xenopus* precedes each inspiration phase with a single expiration, thereby ensuring a more complete renewal of the lung gas store (Brett & Shelton, 1979). Taken together, these factors seem most appropriate to an exclusively aquatic animal whose acquisition of fresh air may become limited to rather brief episodes at the water surface (Boutilier, 1984).

One obvious outcome of intermittent ventilation in *Xenopus* is that it leads to considerable oscillations in the respiratory gas tensions in blood (Emilio & Shelton,

1974; Shelton & Boutilier, 1982). During a dive, however, the changes in P_{CO_2} are far less than those of P_{O_2}, owing to the differences in the capacitances of the respiratory gases in water surrounding the permeable skin. As a result, the animal is able to operate over a wide range of blood P_{O_2}, and thus blood O_2 contents, without becoming severely acidotic (Emilio & Shelton, 1974; Shelton & Boutilier, 1982; Boutilier, 1984). From the oxygen equilibrium curves in Fig. 1, it is clear that the blood O_2 store will change in a complex way throughout the period of diving, as it depends not only on the oxygen utilization by the animal but also on the magnitude of the changes in P_{CO_2} and thus pH (Fig. 2). In addition, the lungs of *Xenopus* are used as an additional storage site for oxygen during a dive and may be selectively perfused so as to supplement an ever decreasing store of blood oxygen (Shelton & Boutilier, 1982). Just as the deoxygenated blood of *Xenopus* may be used as an effective storage site for bulk delivery of carbon dioxide to the lungs during breathing, the comparatively high Bohr effect (Table 1) represents an adaptation which will facilitate the delivery of the blood O_2 store to the tissues during a dive. It seems likely that these biochemical adaptations are evolutionarily linked to the changes in respiratory blood flow during intermittent breathing which are made possible by the undivided ventricle. Thus, the Haldane effect occurs in unison with high blood flow to the lungs (Shelton, 1970), whereas the Bohr effect appears to be linked to an increasing movement of blood into the systemic circulation as apnoea progresses (Shelton & Boutilier, 1982).

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REFERENCES

BAROIN, A., GARCIA-ROMEU, F., LAMARE, T. & MOTAIS, R. (1984). Hormone-induced co-transport with specific pharmacological properties in erythrocytes of rainbow trout, *Salmo gairdneri*. *J. Physiol., Lond.* **350**, 137–157.

BENNETT, A. F. (1978). Activity metabolism of the lower vertebrates. *A. Rev. Physiol.* **400**, 447–469.

BOUTILIER, R. G. (1984). Characterization of the intermittent breathing pattern in *Xenopus laevis*. *J. exp. Biol.* **110**, 291–309.

BOUTILIER, R. G., EMILIO, M. G. & SHELTON, G. (1986). Effects of mechanical work on electrolyte and water distribution in amphibian skeletal muscle. *J. exp. Biol.* **120**, 333–350.

BOUTILIER, R. G., MCDONALD, D. G. & TOEWS, D. P. (1980). The effects of enforced activity on ventilation, circulation and blood acid-base balance in the aquatic gill-less urodele, *Cryptobranchus alleganiensis*: a comparison with the semi-terrestrial anuran, *Bufo marinus*. *J. exp. Biol.* **84**, 289–302.

BOUTILIER, R. G., RANDALL, D. J., SHELTON, G. & TOEWS, D. P. (1977). Some response characteristics of CO_2 electrodes. *Respir. Physiol.* **32**, 381–388.

BOUTILIER, R. G. & TOEWS, D. P. (1981). Respiratory properties of blood in a strictly aquatic and predominantly skin-breathing urodele, *Cryptobranchus alleganiensis*. *Respir. Physiol.* **46**, 161–176.

BRETT, S. S. & SHELTON, G. (1979). Ventilatory mechanisms of the amphibian, *Xenopus laevis*; the role of the buccal force pump. *J. exp. Biol.* **80**, 251–269.

DEJONGH, H. J. & GANS, C. (1969). On the mechanism of respiration in the bullfrog, *Rana catesbeiana*: a reassessment. *J. Morph.* **127**, 259–290.

EMILIO, M. G. (1974). Gas exchanges and blood gas concentrations in the frog, *Rana ridibunda*. *J. exp. Biol.* **60**, 901–908.

EMILIO, M. G. & SHELTON, G. (1972). Factors affecting blood flow to the lungs in the amphibian, *Xenopus laevis*. *J. exp. Biol.* **56**, 67–77.

EMILIO, M. G. & SHELTON, G. (1974). Gas exchange and its effect on blood gas concentrations in the amphibian, *Xenopus laevis*. *J. exp. Biol.* **60**, 567–579.

EMILIO, M. G. & SHELTON, G. (1980). Carbon dioxide exchange and its effects on pH and bicarbonate equilibria in the blood of the amphibian, *Xenopus laevis*. *J. exp. Biol.* **83**, 253–262.

GEVERS, W. (1977). Generation of protons by metabolic processes in heart cells. *J. molec. Cell Cardiol.* **9**, 867–874.

HAAB, P. E., PIIPER, J. & RAHN, H. (1960). Simple method for rapid determination of an O_2 dissociation curve of the blood. *J. appl. Physiol.* **15**, 1148–1149.

HECK, H. D'A. (1970). A general derivation of the binding potential. *J. molec. Biol.* **50**, 703–705.

HOCHACHKA, P. W. & MOMMSEN, T. P. (1983). Protons and anaerobiosis. *Science* **219**, 1391–1397.

JOKUMSEN, A. & WEBER, R. E. (1980). Haemoglobin-oxygen binding properties in the blood of *Xenopus laevis*, with special reference to the influences of aestivation and of temperature and salinity acclimation. *J. exp. Biol.* **86**, 19–37.

JONES, D. R. (1972). Anaerobiosis and the oxygen debt in an anuran amphibian, *Rana esculenta*. *J. comp. Physiol.* **77**, 356–382.

JONES, D. R. & MUSTAFA, T. (1973). The lactacid oxygen debt in frogs after one hour's apnoea in air. *J. comp. Physiol.* **85**, 15–24.

LENFANT, C. & JOHANSEN, K. (1967). Respiratory adaptations in selected amphibians. *Respir. Physiol.* **2**, 247–260.

LILLO, R. S. (1978). The effect of arterial-blood P_{O_2}, P_{CO_2} and pH on diving bradycardia in the bullfrog, *Rana catesbeiana*. *Physiol. Zool.* **51**, 340–346.

MCDONALD, D. G., BOUTILIER, R. G. & TOEWS, D. P. (1980). The effects of enforced activity on ventilation, circulation and blood acid-base balance in the semi-terrestrial anuran, *Bufo marinus*. *J. exp. Biol.* **84**, 273–287.

MACINTYRE, D. H. & TOEWS, D. P. (1976). The mechanics of lung ventilation and the effects of hypercapnia on respiration in *Bufo marinus*. *Can. J. Zool.* **54**, 1364–1374.

NIKINMAA, M. (1983). Adrenergic regulation of haemoglobin oxygen affinity in rainbow trout red cells. *J. comp. Physiol.* **152**, 67–72.

PALFREY, H. C. & GREENGARD, P. (1981). Hormone-sensitive ion transport systems in erythrocytes as models for epithelial ion pathways. *Ann. N.Y. Acad. Sci.* **372**, 291–308.

PERUTZ, M. F. & BRUNORI, M. (1982). Stereochemistry of cooperative effects in fish and amphibian haemoglobins. *Nature, Lond.* **229**, 421–426.

PÖRTNER, H. O., HEISLER, N. & GRIESHABER, M. K. (1984). Anaerobiosis and acid-base status in marine invertebrates: a theoretical analysis of proton generation by anaerobic metabolism. *J. comp. Physiol.* **155B**, 13–20.

REEVES, R. B. (1976). Temperature-induced changes in blood acid-base status: pH and P_{CO_2} in a binary buffer. *J. appl. Physiol.* **40**, 752–761.

SCHEID, P. & MEYER, M. (1978). Mixing technique for study of oxygen-hemoglobin equilibrium: a critical evaluation. *J. appl. Physiol.* **45**, 818–822.

SHELTON, G. (1970). The effect of lung ventilation on blood flow to the lungs and body of the amphibian, *Xenopus laevis*. *Respir. Physiol.* **9**, 183–196.

SHELTON, G. (1976). Gas exchange, pulmonary blood supply, and the partially divided amphibian heart. In *Perspectives in Experimental Biology*, vol. 1, Zoology (ed. P. Spencer Davies), pp. 247–259. Oxford, New York: Pergamon Press.

SHELTON, G. (1985). Functional and evolutionary significance of cardiovascular shunts in the Amphibia. In *Cardiovascular Shunts* (ed. K. Johansen & W. W. Burggren), pp. 100–120. Alfred Benzon Symposium **21**. Copenhagen: Munksgaard.

SHELTON, G. & BOUTILIER, R. G. (1982). Apnoea in amphibians and reptiles. *J. exp. Biol.* **100**, 245–273.

SHELTON, G., JONES, D. R. & MILSOM, W. K. (1985). Control of breathing in ectothermic vertebrates. In *Handbook of Physiology*, American Physiological Society (in press).

TAZAWA, H., MOCHIZUKI, M. & PIIPER, J. (1979). Blood oxygen dissociation curve of the frogs *Rana catesbeiana* and *Rana brevipoda*. *J. comp. Physiol.* **129**, 111–114.

TOEWS, D. P. (1971). Factors affecting the onset and termination of respiration in the salamander, *Amphiuma tridactylum*. *Can. J. Zool.* **49**, 1231–1237.

TOEWS, D. P., SHELTON, G. & RANDALL, D. J. (1971). Gas tensions in the lungs and major blood vessels of the urodele amphibian, *Amphiuma tridactylum*. *J. exp. Biol.* **55**, 47–61.

TURNER, J. D., WOOD, C. M. & HÖBE, H. (1983). Physiological consequences of severe exercise in the inactive benthic flathead sole (*Hippoglossoides elassodon*): a comparison with the active pelagic rainbow trout (*Salmo gairdneri*). *J. exp. Biol.* **104**, 269–288.

WEBER, R. E. (1982). Intraspecific adaptation of hemoglobin function in fish to oxygen availability. In *Exogenous and Endogenous Influences on Metabolic and Neural Control* (ed. A. D. F. Addink & N. Sprank), pp. 87–102. Oxford: Pergamon.

WEST, N. H. & JONES, D. R. (1975). Breathing movements in the frog *Rana pipiens*. I. The mechanical events associated with lung and buccal ventilation. *Can. J. Zool.* **53**, 332–344.

WOOD, C. M., MCMAHON, B. R. & MCDONALD, D. G. (1977). An analysis of changes in blood pH following exhausting activity in the starry flounder, *Platichtys stellatus*. *J. exp. Biol.* **69**, 173–185.

WOOD, S. C., WEBER, R. E., MALOIY, G. M. O. & JOHANSEN, K. (1975). Oxygen uptake and blood respiratory properties of the Caecilian *Boulengerula taitanus*. *Respir. Physiol.* **24**, 355–363.

WOODBURY, J. W. (1974). Body acid-base state and its regulation. In *Physiology and Biophysics*, 20th edn, vol. II (ed. T. C. Ruch & H. D. Patton). Philadelphia: W. B. Saunders Co.

WYMAN, J. (1964). Linked functions and reciprocal effects. *Adv. Protein Chem.* **19**, 223–286.