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RESPIRATORY PROPERTIES OF BLOOD IN A STRICTLY AQUATIC AND PREDOMINANTLY SKIN-BREATHING URODELE, *CRYPTOBRANCHUS ALLEGANIENSIS*

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Abstract. The strictly aquatic urodele, *Cryptobranchus alleganiensis.* is one of the largest gill-less vertebrates in which most of the respiratory gas exchanges occur across the skin. In this study we have examined some of the gas carrying relationships in blood to determine whether certain properties are particularly adaptive to the hellbender"s well oxygenated habitat and predominantly cutaneous mode of respiration. The0_2 dissociation curve is sigmoidal (n = 2.9) having a $\mathbf{P_{50}}$ of 23.6 mm Hg (at pHa and Paco,) and a Bohr factor of -0.24. A considerable amount of arterio-venous mixing prior to the ejection of blood from the heart is thought to account for a comparatively low arterial O_2 saturation (57.5'\ at Pa_{0}• of 27.4 mm Hg). The *in vitro* true plasma buffer value was -12.0 mmo-l1-1- p H- ^{1} for a hemato rit of 29 {,. Attention is drawn to the variability in the protein bufferingrelated CO_2 combining properties *in vitro* caused by hct alterations during different methods of bloodsampling. Acid-base relationships between whole blood, true plasma and separated plasma are essentially the same as those described for mammalian blood.

Interspecies comparisons with the data from this study suggest that factors such as microhabitat, rather than water or air breathing *per se,* may influence the characteristics of blood 0_2 and CO_2 carriage in amphibians.

Blood	Haemoglobin
Bohr effect	P_{50}
Carbon dioxide absorption	Skin breathing

Cryptobranchus alleganiensis, commonly called the hellbender, is one of the largest representatives of the North American urodeles with reported lengths of up to 74 cm and weights of over a kg (Nickerson and Mays, 1973). Animals are found in clear fast-flowing streams of the eastern United States and while there is a long standing

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literature *(i.e.* Harlan, 1825) on their natural history and habits (reviewed by Nickerson and Mays, 1973), only recently has the dominance of the skin in overall gas exchange been clearly defined.

Guimond and Hutchison (1973) have shown that cutaneous respiration in *Cryptobranchus* accounts for 90% $\dot{V}_{O_2}$ and 97% $\dot{V}_{CO_2}$ at temperatures ranging from 5-25 °C. Moreover, the animals can be forcibly submerged for several weeks (Guimond and Hutchison, 1976) or have their lungs surgically removed (Robin in Hughes, 1967) without any apparent adverse effects to gas exchange. Notwithstanding the poorly vascularized and non-septate structure, the lungs do occupy a considerable volume in the resting animal and are ventilated at increased frequencies in response to a rise in tissue CO_2 output (Boutilier *et al.,* I 980) or environmental hypercapnia (Boutilier and Toews, 1981).

Morphological adaptations for cutaneous gas exchange are numerous and include a richly vascularized integument with highly superficial capillaries particularly in the regions of loose reticulated skin which hang from the lateral margins of the body wall and legs (Noble, 1925; personal observations). These skin folds together with a dorso-ventrally flattened body form serve to increase the functional surface areas for respiratory gas exchange. Even though the animals can survive in a moisture saturated aerial environment for periods of weeks or more (Guimond and Hutchison, 1976) they are reported, with rare exception, as being strictly aquatic in their natural habitat (Nickerson and Mays, 1973).

Certainly, this animal must be one of the largest aquatic vertebrates in which almost all of the gas exchange is cutaneous. As such, *Cryptobranchus* is not subject to the ventilatory limitations associated with aquatic breathing (Rahn, 1966) but rather on diffusion and perfusion relationships of the skin (Piiper *et al.,* 1976) and the gas carrying properties of the blood.

Because there is a documented tendency for certain of the blood respiratory properties of amphibians - such as P_{50}, Bohr effect and buffering capacity - to be positively correlated with an increasing dependence on pulmonary ventilation (Lenfant and Johansen, 1967; Johansen and Lenfant, 1972), the present study investigates whether such relationships hold for an animal at the strictly aquatic end of the water to air-breathing spectrum.

Materials and Methods

Cryptobranchus a. alleganiensis (400-925 g) were collected in Missouri and air-shipped to Nova Scotia where they were maintained in well aerated conditions at 25 ± I °C. Arterial catheters (p.e. 60) were chronically implanted in the truncus arteriosus and animals were allowed 24 h recovery before blood sampling (detailed in Boutilier *ct al.,* 1980). In other instances, large blood samples (10-12 ml) were drawn from the con us arteriosus of lightly anaesthetized animals and stored at 5 °C pending *in vitro* analysis. Blood drawn from the catheters (up to 2 ml per sample)

of resting animals was used immediately for *in vivo* and *in vitro* study. Because the large red cells (35-45 µm) tended to settle out very rapidly, it was necessary to thoroughly mix the parent blood sample to insure similarity of haematocrit aliquots. Carbon dioxide combining curves (Ceo, *vs* P co,) and buffer lines ([Heon *vs* pH) were constructed for oxygenated and deoxygenated samples of whole blood. Aliquots of blood (0.2-1.0 ml) were equilibrated in round bottomed tonometers against humidified gas mixtures (from Wosthoff pumps) containing various percentages of CO_2, the balance of which was either air or nitrogen. Following the equilibration period (15-30 min) samples were taken up in gas-tight syringes and anaerobically introduced into pH and Ceo, electrode chambers. Replicate haematocrit determinations accompanied each sample. The equilibration protocol followed two directions: large samples (1-2 ml) were either equilibrated continuously, with sampling occurring 15-30 min after each step increase in Pco,, or fresh aliquots (200---400 µI) from a single refrigerated blood pool were introduced each time the Pco, was changed. The latter method was favoured after it was found that long term tonometry produced spurious results, particularly for oxygenated and deoxygenated whole blood comparisons (see Results).

CO_2 combining curves and buffer lines were also constructed for oxygenated samples of separated plasma and true plasma using the same equilibration methods. True plasma was obtained by equilibrating whole blood samples with the various CO_2 gas mixtures and then anaerobically centrifuging off the plasma (5000 g for 3 min, Beckman microfuge 0.4 ml sealed tubes) for measurements of pH and Ceo,· The whole blood and separated plasma corresponding to each true plasma sample was also analyzed for pH and Ceo,.

The solubility of CO_2 in *Cryptobranchus* plasma (Cco,/PcoJ was determined after acidification of separated plasma with lactic acid (Van Slyke *et al.*, 1928) and equilibration with 100% CO_2 at 25 °C (N = 5). The first dissociation constant of carbonic acid (pK;) was then estimated gasometrically as described in previous papers (Truchot, 1976; Boutilier *et al.*, 1979).

Oxygen equilibrium curves were constructed using a mixing technique whereby various oxyhaemoglobin saturations are prepared for P_0, and pH measurement by anaerobically mixing known proportions of oxygenated and deoxygenated whole blood. Aliquots of each blood sample having the same **P**co, were taken up in the various proportions using a single I-ml gas-tight Hamilton syringe (volumes determined by syringe barrel graduation) and were thoroughly mixed by gentle agitation with a bead of mercury which had initially filled the syringe deadspace.

Measurements of **pH, P**co,, Ceo,, and $\mathbf{P}_{02}$ were made using Radiometer electrodes and display meters, the analytical techniques of which have been detailed previously (Boutilier *et al.*, 1979, 1980; McDonald *et al.*, 1980). Bicarbonate concentrations in whole blood and true plasma were calculated from measured values of Ceo,, and IXc0, using the equation; $[HCO_3^-] = Ceo, - etco, \cdot Pco,$. Chloride concentrations were measured with a Buchler-Cotlove chloride titrator. [Heon and[Cl -1 within the erythrocyte were estimated from their respective concentrations in whole blood

and true plasma according to the formula:

$$[E\dagger = \frac{[BJ - [P](1-Hct)}{Hct}$$

where all concentrations are expressed per kilogram of red cell or plasma water (0.895±0.017 kg $H_2O \cdot I^{-1}$ red cells; 0.967±0.005 kg $H_2O \cdot 1^{-1}$ plasma). Water contents in whole blood of known hct and in plasma were determined on 1 ml samples which were weighed, vacuum dried for 24 h (60 °C) and then reweighed. All hct measurements (at least 3 per sample) were made immediately following 3 min of centrifugation (3000 g) in a Clay-Adams microfuge. It was earlier checked that longer periods of centrifugation gave the same hct values. Precautions were taken to insure that the blood samples remained anaerobic during injection and centrifugation. All hct measurements were made at physiological Paco, (1% CO_2) unless otherwise indicated.

Results

O_2 COMBINING PROPERTIES OF WHOLE BLOOD

Oxygen dissociation curves and Bohr effect were studied on independent whole blood samples from six *Cryptobranchus* (fig. 1). When equilibrated with 1 % C O_2 gas mixtures (P*co*, 7.4 mm Hg), the mean $\mathbf{P_{50}}$ of the averaged curves of five animals was 23.6 mm Hg (range, 22-25.5 mm Hg; fig. 1). Mean values of the acid-base variables measured on $\mathbf{P_{50}}$ blood samples (shown in fig. 1) are in accord with *in vivo* measurements from resting catheterized animals at 25 °C (table 1). When corresponding *in vivo* arterial blood $\mathbf{P}_{02}$ values (N = 7; Boutilier and Toews, 1981) are plotted directly on the dissociation curve in fig. 1, their mean corresponds to an arterial blood $\mathbf{0}_2$ saturation of 57.5% (range, 52-68'.1/,,).

Blood from six animals exhibits a CO_2 related Bohr effect with a slope of -0.24 (fig. I). Hill's cooperativity coefficient n, assessed in the *20'.1/,,* to 90% 0_2 saturation range, was 2.9 (table I).

CO_2 COMBINING PROPERTIES OF WHOLE BLOOD AND PLASMA

A selected group of four CO_2 combining curves, each determined at 25 °C on oxygenated whole blood of individual hellbenders, exhibit considerable variation in shape and position (fig. 2). Over the physiological range of Paco, (4-8 mm Hg, table I), the slopes of these curves can be quantified by the capacitance coefficient, $PCO_2 = LICco,ILIPco,$ (Piiper *et al.*, 1971), which when graphically estimated for the data in fig. 2, shows that the higher the hct, the greater is the coefficient. This necessarily reflects the positively correlated relationship between blood

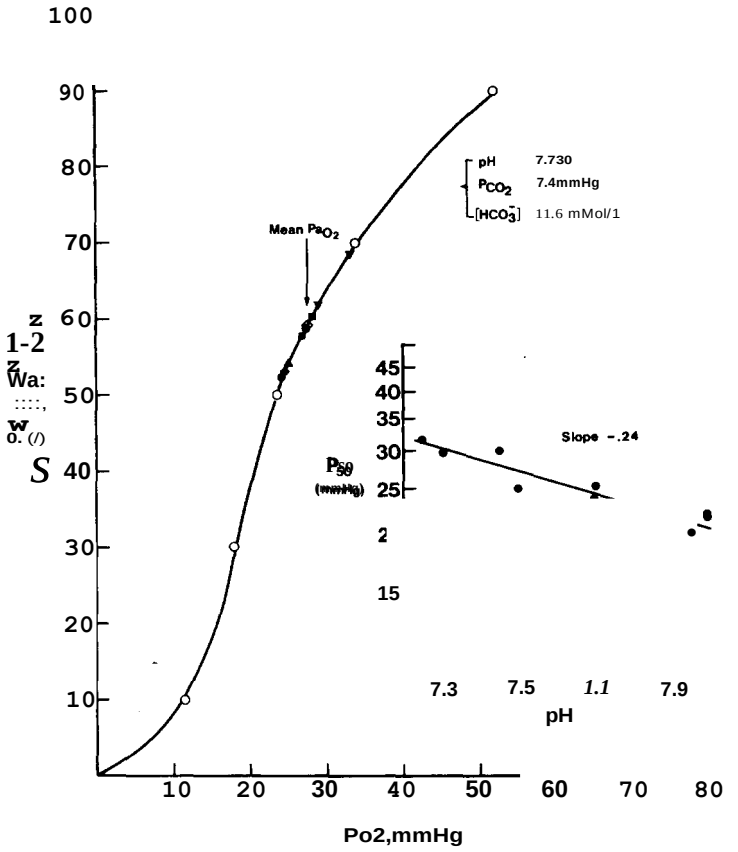

Fig. I. Mean 0_2 dissociation curve (open circles; N = 5) and Bohr effect (N = 6) in *Cryptobranchus* whole blood. All *in vitro* analyses performed on blood drawn from resting catheterized animals. PH, Pco, and $[HCO_3]$ are mean values corresponding to P_{50} samples of averaged 0_2 curves. *In vivo* Pa_{0_2} values of seven *Cryptobranchus* (data from Boutilier and Toews, 1981) are shown by different symbols between 50 and 70% 0_2 saturation. Mean Pa_0, "'mean 0_2 saturation of 57.5%. All measurements made at 25 °C.

buffering capacity ($\Delta HCO_3 / \Delta pH$) and hct previously reported (Boutilier *et al.*, 1980). The CO_2 dissociation curves also differ in their overall vertical positions on the plot (fig. 2) which depend on the metabolically fixed concentrations of *Ceo,* in the blood at the time of its withdrawal *in vivo* (irrespective of Paco,) and the haematocrit.

The high hct bloods in fig. 2 (hct 36, 40) were obtained from anaesthetized animals (see Methods) while bet's 25 and 31 were for blood samples drawn from the cannulae of resting undisturbed hellbenders. Hct differences of this order were always found between the two blood sampling methods with blood from anaesthetized salamanders producing comparatively steeper CO_2 dissociation curves and higher blood buffering capacities (table I).

TABLE I

In vivo and *in vitro* respiratory characteristics of *Cryptobranchus* whole blood and plasma at 25 °C. Values are means ± standard error of the mean where indicated. Cannulated and anaesthetized animals refer to the state of the salamander when the blood samples were collected.

P_{50} (mm Hg)	23.6 ± 1.4
Hill's coefficient, n	2.9
Bohr effect ($\Delta \log P_{50} \cdot \Delta pH^{-1}$)	-0.24
Arterial O_2 saturation (%)	57.5
Pa_{O_2} (mm Hg)*	27.4 ± 0.9
Paco, (mm Hg)*	6.2 ± 1.4
pHa*	7.786 ± 0.022
[Cco,la (mmol $\cdot l^{-1}$)*	8.31 ± 1.86
Plasma [HCO_3^- la (mmol $\cdot l^{-1}$)*	10.99 ± 0.76
[Lactatela (mmol $\cdot l^{-1}$)**	0.56 ± 0.10
Buffer value, true plasma ($\Delta[HCO_3] \cdot \Delta pH^{-1}$)	
Cannulated animals	-12.0 ± 2.5
Anaesthetized animals	-18.6 ± 3.2
Haematocrit (percent)	
Cannulated animals	29.3 ± 2.1
Anaesthetized animals	36.5 ± 1.6
Plasma pK_1'	6.17 ± 0.05
CO_2 in plasma (mmol $\cdot l^{-1} \cdot mm\ Hg^{-1}$)	0.043 ± 0.003

* Data from Boutilier and Toews (1981), **N** = 7.

** Data from Boutilier *et al.* (1980), **N** = 8.

CO_2 combining curves for oxygenated and deoxygenated whole blood comparisons were determined on four occasions. A Haldane effect was always present (1.5-2.0 mmol/l *Ceo,;* 0.05-0.13 pH units difference) when freshly drawn blood samples were equilibrated in tonometers for not more than 30 min. Longer term tonometry produced uncontrolled metabolic activity in the deoxygenated blood resulting in a steady depression of blood *Ceo,* and pH with time.

A family of CO_2 dissociation curves for oxygenated whole blood, true plasma and separated plasma from a single animal (hct = 30%) are illustrated in fig. 3 and correspond in pattern to the trends seen in one additional independent series of this sort. *Ceo,* differences between the two plasmas are determined by **RBC** presence or absence, however, at the Pco, tension where centrifugation for separated plasma originally took place, the *Ceo,* concentrations are by definition equal. The reason for the high **P***co,* of separation (14 mm Hg, fig. 3), relative to Paco, (table I), is that the blood for this experiment was taken from an anaesthetized animal (sample centrifuged immediately upon withdrawal) thereby illustrating the large respiratory acidosis accompanying this method of blood collection. Differences in shape *(i.e. PCOi)* between the CO_2 curves in fig. 3 are related to the buffering capacity of the respective fluids (fig. 4).

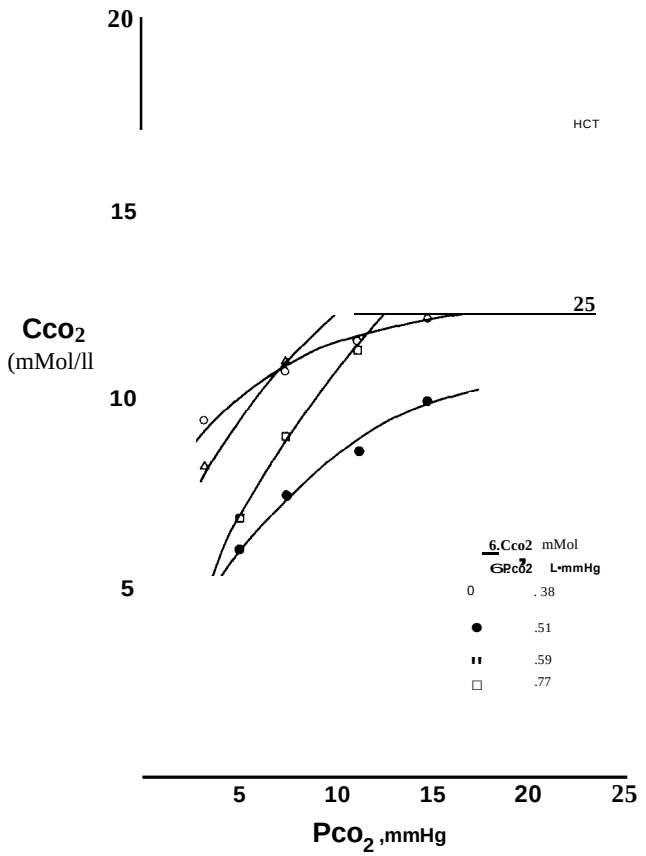

Fig. 2. Oxygenated whole blood CO_2 combining curves of four individual *Cryptobranchus* (25 °C) showing effects of haematocrit on curve position and slope *(i.e.* capacitance coefficient, *LI Ceo,!* LIPco, for Pco, range of 4--8 mm Hg). Blood drawn from anaesthetized (hct's 36, 40) and resting catheterized (hct's 25, 31) animals.

Because the intracellular to extracellular concentration ratios for $HC0_3^-$ and C-l are equal ('[$HC0_3^-$ J = '[C-l], table 2), we can assume the existence of a Gibbs-Donnan equilibrium for these ions. Analysis of true plasma [Cl-J during both series of *in vitro* CO_2 equilibrations *(i.e.* figs. 3 and 4) reveals that the amount of RBC bicarbonate added to the plasma (ACco, True-ACco, Sep. for given APcoJ is stoichiometrically equivalent to plasma C-l loss (fig. 5). In these respects, the blood of *Cryptobranchus* behaves as mammalian blood.

As a corollary to the chloride shift (fig. 5), the replacement of the haemoglobin negative ions by c1- and $HC0_3^-$ will lead to an increase in the total number of osmotically active ions within the RBC. The resultant difference in osmotic pressure will be reconciled by a migration of water from plasma to cells (i.e. cell swelling). This appears to be the case in *Cr;ptobranchus* since data which correspond to the

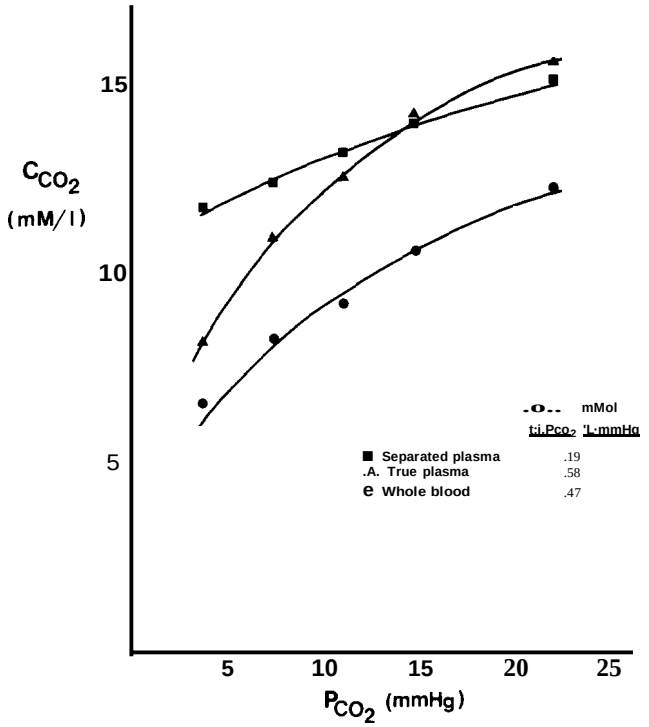

Fig. 3. CO_2 combining curves and respective capacitance coefficients (LIC*co,!*LIP*co)* for oxygenated whole blood **(e),** true plasma (.&) and separated plasma (■) determined *in vitro* at 25 °C. Analyses made on terminal blood sample drawn from an anaesthetized animal. Dashed line is the solubility of CO_2 in separated plasma.

TABLE 2

Concentration of chloride and bicarbonate in plasma (p) and erythrocytes (e) of blood samples (hct = 0.29 ± 0.02) drawn from resting undisturbed *C'1)ptobranchus* (N = 6). All concentrations are expressed in mequiv • kg -I of cell and plasma water respectively (x ± I S.E.M.). r = Donnan ratio = [erythrocyte];[plasma].

[HC0 $_3$- Je	5.46 ± 0.93	[Ci-Jc	37.83 ± 4.07
[H C0-$_3$ p]	11.11 ± 0.87	(Ci-Jr	73.59 ± 2.34
r[HCOn	0.49 ± 0.o7	'[Ci-]	0.51 ±0.05

latter experiments (figs. 3-5) show that hct increases as **P**co, levels rise (fig. 6). Because the effect is reversible (open symbols, fig. 6) we can attribute the hct increases to red cell swelling as a result of the aforementioned interactions between **RBCs** and plasma.

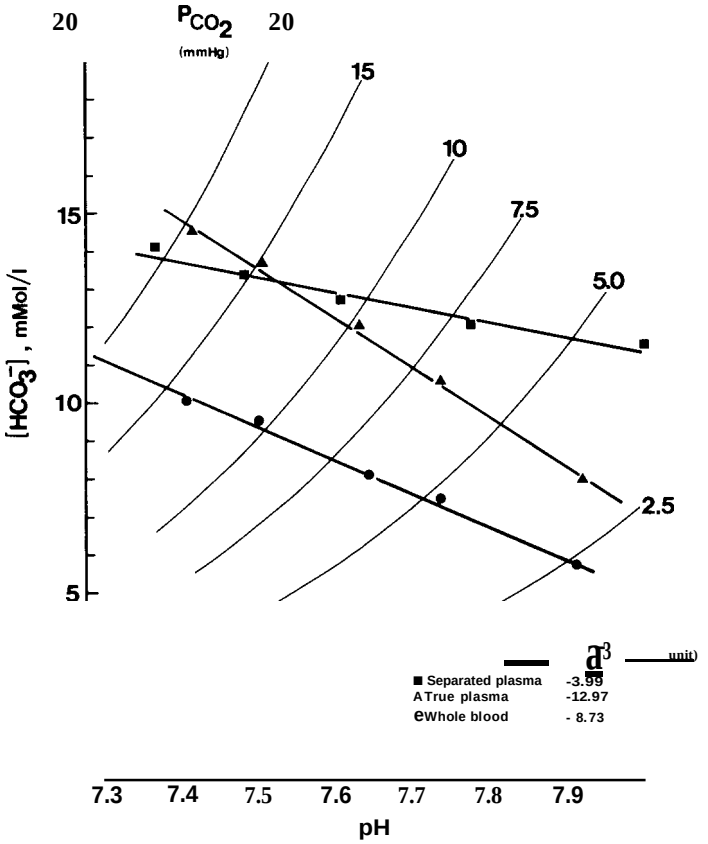

Fig. 4. Buffer lines and corresponding buffering capacities (ΔHCO_3^- / ΔpH) for oxygenated whole blood (●), true plasma (▲) and separated plasma (■) determined *in vitro* at 25 °C at the same time as data in fig. 3. Lines fitted and slopes determined by least squares regression analysis.

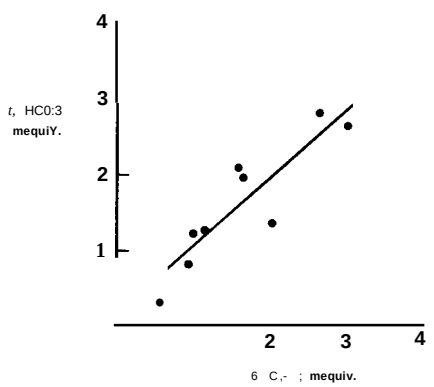

Fig. 5. Relationship between amount of bicarbonate added to plasma and amount of plasma chloride lost to red cells during oxygenated blood CO_2 equilibrations shown in figs. 3 and 4 plus one additional independent series. Large changes represent reequilibrations from high to low Pco, levels. All concentrations corrected to mequiv, kg^{-1} of plasma water. Least squares regression line drawn through points ($y = 0.89x + 0.20$) has correlation coefficient of 0.91.

TABLE 3

A comparison of gas carrying properties of the blood of species from the three orders of Amphibia. Values are means.

Species	Principal organ of gas exchange	Pso (mm Hg)	Hill's n	Bohr effect (,'llog P50 · .1pH-^{1})	Conditions of *02* curve construction		*/11 vivo* and *in vitro* Temp. eci
					pH	Pco, (mm Hg)	
URODELA:							
Cr.1ptohranchus a. alleraniensis	skin	23.6	2.9	-0.240	7.730	7.4	25
Necturus maculosus	gill	14.5	1.4	-0.131		6.0	20
Amphiuma tridactylum	lung	27.0	1.95	-0.205		8.5	**22**
Desmognathw /Uscus	skin	27.0	2.64		7.5	7.4	13
ANURA							
Rana catesheiana	lung	39.0	1.95	-0.288		10.0	22
Bu/a ***rnarinus***	lung	53.0	2.78	-0.230	7.750	14.8	25
Tc/matohius culeus	skin	15.6	2.0	-0.30			10
APODA							
Typh/onectes ***con1pressicauda***	lung	22.6	2.15	-0.078	7.620	I7.1	30
Boulengcrula ***taitanus***	lung	270	1.79	-0.21	7.60		25

* Estimates from available data.

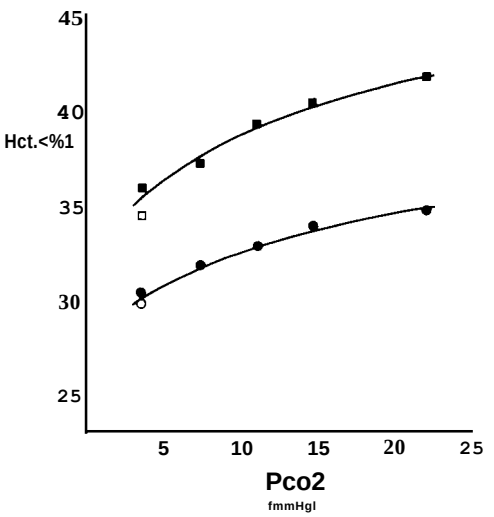

Fig. 6. Relationship between haematocrit and blood Pco, during *in vitro* step Pco, equilibrations **(N** = 2) at 25 °C. Lower curve is data collected simultaneously with those appearing in figs. 3-5. Upper curve is that of additional independent series. Open symbols represent re-equilibrations from highest

tn. lrnuPct P

Measured respiratory parameters for arterial blood			Arterial 02 Saturation (";)	Arterial plasma [HCO3-] (mmol -1- 1)	Hct. ("c)	True plasma Buffer Value (.1[HC03-]·.1pH-I)	References
pH	Pco, (mm Hg)	Po, (mm Hg)					
7.786	6.2	27.4	57.5	10.99	29.3	-12.00	This study. Boutilier and Toews (1981)
	4.4	35.2	87*	12*	19	- 8.0	
	6.1	81.2	92*	14.5*	23	- 9.2	Lenfant and Johansen, 1967
7.51	6.2	40.0	70	9.15	28	-13.2	Gatz *et al.*. 1974
	8.2	94.6	92*	17.0*	23.5	-16.4	Lenfant and Johansen (1967)
7.790	12.1	96.0	88	22.3*	25.0	-20.4	Boutilier and Toews, 1977; Boutilier *et al.*, 1979; unpublished results
7.63	8.0			11.7*	23.6	- 8.9	Hutchison *et al.*. 1976
7.55	17.5	55	85	15.0	37.6	- 5.4	Toews and Macintyre. 1977, 1978
					40	-17.8	Wood *et al.*. 1978

CRITIQUE OF METHODS

The mixing technique for determining 0_2 dissociation curves, developed by Haab *et al.* (1960), has been critically examined in a recent paper by Scheid and Meyer (1978) who suggest that the fractional blood volumes are more precisely determined by weight rather than by the volumetric procedures used herein. In addition, we have made no allowances for the possibility that the P_{0_2} of air does not provide complete 0_2 saturation (less than 99% in human blood; Roughton and Severinghaus, 1973), however, this may be of lesser importance than in the lower affinity bloods of birds and mammals (Scheid and Meyer, 1978). Finally, the Pa_{0_2} measurements plotted to give arterial 0_2 saturation estimates in fig. 1 may have revealed differences in 0_2 content *(i.e.* hct variation) but unfortunately these measures are lacking.

Short term tonometry (15-30 min) of fresh blood samples (oxygenated and deoxygenated) or those drawn from a refrigerated blood pool avoided the problems of uncontrolled changes associated with prolonged tonometry. Longer term tono-

metry (step increases in PcoJ was, however, utilized for all oxygenated CO_2 dissociation curves and buffer lines since re-equilibration to low Pco, levels in these experiments showed that no serious metabolic changes (assessed by pH and Ceo,) or hydration/dehydration effects *(i.e.* fig. 6) had occurred.

Haematocrit measurements may have been more precisely determined by taking account of trapped plasma during red cell packing, particularly for the estimates of red cell $HC0_{-1}$ and c1- concentrations. Because the initial intention of this study was directed more toward interspecies relationships, there are certain parameters other than hct which would have provided a more precise account of the non-bicarbonate buffering system (namely the total blood and plasma prptein concentrations and [Hb]).

The importance of chronic cannulation methods for determining blood acid-base variables from resting undisturbed animals have been emphasized by many authors. What seems of equal importance is that blood for *in vitro* analysis be obtained with the same degree of care. For example, the utilization of different blood sampling methods in this study (anaesthetization *vs.* chronic cannulation) resulted in large variations in hct- *(i.e.* protein) related acid-base parameters (table 1, fig. 2). In an earlier study on *Bufo marinus* (Boutilier *et al.,* I 979), the *in vitro* CO_2 content of plasma was markedly reduced, relative to *in vivo* data on catheterized animals, by the appearance of metabolic acids during unacceptable blood sampling techniques (i.e. pithing). It is not difficult therefore to envisage a situation whereby blood sampling techniques alone preclude meaningful interpretations of blood gas transport *in vivo.*

Discussion

The high 0_2- Hb affinity of *Cryptobranchus* blood (fig. 1) functionally serves in establishing a large 0_2 diffusion gradient across the skin thereby facilitating 0_2 loading by epithelial blood. A gradient of this magnitude must be regarded as essential for a strictly aquatic animal in which 90% of its V_{0_2} is cutaneous (Guimond and Hutchison, 1973) and which lacks any specialized mechanisms of convection, relying instead on passive forms of ventilation provided by either water currents or body movements. One characteristic behaviour, when *Cryptobranchus* is confined to poorly aerated water, is body movements whereby the animal rocks or sways *in a* lateral fashion causing the skin folds along the body margin to undulate. Presumably these movements disturb the boundary layer between skin and water renewing at least some of the water immediately surrounding the animal.

Cryptobranchus can support a respiring tissue mass of up to a kilogram or more and while its metabolic rate is amongst the lowest reported for any amphibian (Guimond and Hutchison, 1973, 1976), the low respiratory surface area to volume ratio must limit the transport of oxygen to the interior. Certainly then, the substantial Bohr effect together with the high cooperativity of the blood 0_2

dissociation curve (fig. 1, table 1) must be particularly beneficial in facilitating 0_2 unloading at the tissue level. A similar situation in aquatic breathing amphibians which inhabit 0_2 poor environments (i.e. *Necturus,* table 3) would be clearly disadvantageous since it would at the same time impair the 0_2 loading capabilities by functionally reducing the 0_2 diffusion gradient between ambient water and blood at the respiratory exchange site.

From a comparative viewpoint, the high degree of sigmoidicity of the hellbender 0_2 dissociation curve is seen as a clear divergence from that of another aquatic but gill-breathing urodele, *Necturus* (Lenfant and Johansen, 1967; table 3). McCutcheon and Hall (1937), working on haemoglobin solutions (pH 7.38) from a variety of amphibians, have already commented on the 'exaggerated sigmoid shape' of the *Cryptobranchus* 0_2 curve as offering a combination of loading and unloading capacities characteristic of both aquatic and terrestrial amphibians. Certainly the high n value and presence of a considerable Bohr effect are in opposition with the documented trends for aquatic breathers in general (Johansen and Lenfant, 1972). Furthermore, the 0_2- Hb affinity of *Cryptobranchus* is not unusually high *(i.e. Necturus* and perhaps *Telmatobius* are higher) being on par with that of a predominantly air breathing but fossorial apodan, *Typhlonectes* (Toews and Macintyre, 1978; table 3). All of the latter *(Necturus, Telmatobius* and *Typhlonectes),* be they more aquatic or aerial in their mode of 0_2 exchange, habitually endure either low or fluctuating levels of oxygen in their principal respiratory medium where a high 0_2- Hb affinity is obviously an adaptive advantage. On the other hand, field studies on the geographically restricted populations of *Cryptobranchus* (Nickerson and Mays, 1973) suggest that oxygen is rarely limiting in their natural environment. Further speculations about relationships between blood0_2 properties and microhabitat are tempting but probably unwarranted. It is, however, obvious (data in table 3) that the mode of breathing *per se* does not necessarily predetermine the characteristics of 0_2- Hb affinity or overall 0_2 carriage by the blood.

Separation of blood streams from the auricles through the undivided amphibian ventricle is thought to be accomplished by a pressure differential mechanism (Toews, 1971) and/or by the presence of a spiral valve located in the con us arteriosus (Shelton, 1976). *Cryptobranchus,* however, lacks a similar valve structure and moreover, has a largely perforated atrial septum (Guimond and Hutchison, 1973). In addition, blood which leaves the heart does so through a large single vessel (truncus arteriosus), samples from which probably represent a considerable mixture of oxygenated and deoxygenated blood. This blood necessarily perfuses all systemic circuits and must in part be responsible for the low arterial 0_2 saturation (fig. I, table 1). In comparison to other amphibian species, *Cryptobranchus* has the lowest ..-terial0_2 saturation yet reported (table 3), seconded only by that of another skin breathing urodele, *Desmognathus,* which like the hellbender is thought to lack a mechanism for the selective distribution of blood (Gatz *et al.,* 1974).

Arterial blood P*co,* of *Cryptobranchus* at 25 °C (table I) pivots upon the

theoretical maximum predicted for water breathing vertebrates (Rahn, 1966). The presumed mixing of oxygenated and deoxygenated blood apparently accounts for the high Paco, in the same way it affects arterial O_2 saturation. Although little is known about the mechanisms which control CO_2 losses in skin breathing animals, the interactions between RBC and plasma (figs. 3-6) suggest that transcutaneous CO_2 exchange probably follows a conventional vertebrate pattern whereby plasma bicarbonate is dehydrated after movement into the RBC in exchange for chloride *(i.e.* chloride shift, fig. 5). Clearly, more information is needed on the time constant of the shift and the contact time for the blood at the site of gas exchange *(i.e.* cutaneous perfusion) before the physiological significance can be precisely defined. Recent evidence on *Cryptobranchus* does suggest, however, that skin CO_2 loss is poorly controlled (Boutilier *et al.,* 1980) and fluctuates primarily with metabolic rate, whether animals have access to air breathing or not (Moalli *et al.,* 1981).

Relative to other amphibian species, the arterial blood Pm, of *Cryptobranchus* is slightly higher than that of the gill-breathing *Necturus* but similar to that of the obligate air breathing urodele, *Amphiuma* (table 3). At 13 °C, the Paco, of the exclusively skin breathing but predominantly terrestrial plethodont, *Desmognathus,* equals that of the hellbender, however, it is probably higher at a comparable temperature (Moalli *et al.,* 1981 report a Paco, of 4.2 mm Hg for *Cryptobranchus* at 15 °C). That aquatic amphibians have generally lower Paco, levels (Lenfant and Johansen, 1967; Robin *et al.,* 1969; Johansen and Lenfant, 1972) is borne out by the data in table 3. As more data are accumulated, however, some of the interspecific relationships *(i.e.* O_2- Hb affinity, Hill's n and buffering capacity) seem less well matched to mode of breathing and rather more towards adaptations to specific microhabitats; the well oxygenated habitat of *Cryptohranchus* and the hypoxic and/or hypercarbic environment of the fossorial apodan, *Typhlonectes,* being two cases in point. Finally, a more detailed look at intraspecific variability, whether methodological *(i.e.* methods of blood sampling) or true, may help to focus some of the more broad phylogenetic interpretations which are being based on inter-species differences alone.

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