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RESPIRATORY, CIRCULATORY AND ACID–BASE ADJUSTMENTS TO HYPERCAPNIA IN A STRICTLY AQUATIC AND PREDOMINANTLY SKIN-BREATHING URODELE, *CRYPTOBRANCHUS ALLEGANIENSIS*

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Abstract. Upon initial exposure to increased ambient CO_2, *Cryptobranchus* is titrated along an *in vivo* buffer line whose slope is considerably reduced from that observed when whole blood samples are equilibrated *in vitro*. During this time, there is no apparent reduction in the P_{CO_2} difference between arterial blood and inspired media ($Pa_{CO_2} - PI_{CO_2}$), despite an increase in auxiliary respiratory activities (lung and buccopharyngeal ventilation). The development of this non-compensated respiratory acidosis in the skin-breathing salamander is reminiscent of the situation seen in gill-breathing fish where the control of the acid–base balance is achieved by means other than ventilation. The increased ventilatory activities in *Cryptobranchus* can be interpreted as a response to the effect that the acidotic conditions have on arterial oxygenation (*i.e.*: CO_2 Bohr effect); as a result, Pa_{O_2} increases and appears to counteract the arterial hypoxaemia which would otherwise result.

More prolonged hypercapnia leads to a compensatory phase of acid–base adjustment whereby plasma bicarbonate increases along a gently rising Pa_{CO_2} line to a new steady state equilibrium. This compensatory stage is slow acting and offers little by way of restoring the arterial blood pH, at least over the 36-h CO_2 exposure period studied. The recovery period in air-saturated conditions is very gradual with Pa_{CO_2} levels exhibiting an exponential pattern of decline. This, together with the $Pa_{CO_2} - PI_{CO_2}$ observations above, lends support to an accumulating body of evidence which suggests that respiratory CO_2 losses across the amphibian skin are passive or at best only poorly controlled.

Acid–base balance	Hypercapnia
Blood	Respiratory acidosis
Buffer values	Skin breathing

It has long been recognized that the cutaneous surfaces of amphibians provide a major pathway for the elimination of metabolically produced carbon dioxide (Krogh, 1904). However, far less is known about how the skin functions as a respiratory CO_2 exchanger.

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Semi-terrestrial anuran species such as the bullfrog (*Rana catesbeiana*) and toad (*Bufo marinus*) respond to respiratory acid–base disorders brought on by elevated aerobic metabolism (Gottlieb and Jackson, 1976; Mackenzie and Jackson, 1978; Boutilier *et al.*, 1979b; McDonald *et al.*, 1980) or hypercapnia (Macintyre and Toews, 1976; Boutilier *et al.*, 1979a; Jackson and Braun, 1979) by increasing the pattern and frequency of ventilations so as to regulate arterial blood P_{CO_2} and thus pHa. However, the additional data for the bullfrog (Jackson and co-workers) indicate that while Pa_{CO_2} is being controlled by pulmonary ventilation, the corresponding CO_2 losses at the skin proceed in a uniform fashion according to the proportionality constant relating transcutaneous CO_2 diffusion with the ΔP_{CO_2} across the skin (*i.e.* constant skin CO_2 conductance). It could be argued that in a more dynamic situation, such as prolonged diving apnea, comparatively greater perfusion adjustments (*cf.* Shelton, 1970) might exert a more marked effect towards promoting CO_2 losses. However, Pa_{CO_2} levels increase quite substantially during diving behaviours in various anurans (Lenfant and Johansen, 1967; Emilio, 1974; Lillo, 1978; Emilio and Shelton, 1980) and the resultant respiratory acidosis remains uncompensated until the animal resumes its lung ventilatory activities upon surfacing. Thus, in the absence of the regulatory influence of pulmonary ventilation, the skin of anuran amphibians appears to be ineffectual in correcting respiratory acid–base disturbances.

In view of these apparent constraints, one questions the degree of homeostatic control which could be achieved by animals which respire in a predominantly cutaneous mode. Because virtually all CO_2 elimination occurs across the skin of *Cryptobranchus* (Guimond and Hutchison, 1973), respiratory acid–base adjustments will primarily involve transcutaneous diffusion and perfusion relationships (see Piiper *et al.*, 1976 for discussion). In previous studies on this species, in which Pa_{CO_2} increased as a result of elevated aerobic metabolism during activity, we were unable to detect any active form of 'ventilatory' compensation for the attendant respiratory acidosis even though the frequency of lung ventilations increased (Boutilier *et al.*, 1980). More recently, Moalli *et al.* (1981) observed a characteristic ectothermic acid–base response to temperature in *Cryptobranchus* suggesting the process to be an essentially passive result of the thermal effects on metabolic CO_2 production and arterial P_{CO_2} and a temperature-independent CO_2 conductance of the skin.

It became evident that exposing *Cryptobranchus* to environmental hypercapnia would allow us to further investigate the ventilatory response seen earlier (Boutilier *et al.*, 1980) and also to examine whether or not any immediate or long term compensatory processes are actively involved in the respiratory regulation of arterial blood acid–base balance. In addition, our preceding study (Boutilier and Toews, 1981) indicated that a hypercapnic acidosis might also provoke an O_2 respiratory response to counteract the effects that a Bohr shift would bring to arterial blood oxygenation.

Materials and methods

EXPERIMENTAL ANIMALS AND PREPARATION

Male and female specimens of the hellbender, *Cryptobranchus a. alleganiensis* (Daudin), weighing 300–850 g, were collected in Missouri river drainages and air-shipped to Nova Scotia soon after capture. Animals were maintained for several weeks in well aerated water tanks (25 ± 2 °C) and were apparently healthy at the time of experimentation.

Arterial (truncus arteriosus) and buccal catheters were chronically implanted after the salamanders had been anaesthetized by immersion in a 0.035% solution of neutralized (pH 7.6–7.8) MS-222. Following the operative procedures (as in Boutilier *et al.*, 1980), animals were transferred to air-saturated water in the experimental chamber (25 ± 1 °C) where they were allowed to recover and adjust to the apparatus for at least 24 h before measurements began. The chamber consisted of a 120-l aquarium with a plexiglass hood which fit inside to make a water seal at the bottom. Holes in the hood allowed passages for gases and cannulae. Gas distribution tubes positioned in each corner of the chamber water were fed from Wösthoff gas-mixing pumps. Depending on the size of the animal, the water level was adjusted (5–7 cm) to allow ease of access to the aerial compartment. The overall dimensions of the enclosure allowed for unrestrained movements throughout all experiments.

EXPERIMENTAL PROTOCOL

Following post-operative recovery, an additional 12-h period of normoxic normocapnia provided time for blood samples and cardiorespiratory records (3–4 h) to be taken from animals which were resting quietly. A 2.5% CO_2–97.5% air mixture was then bubbled into the chamber water and further data were taken over a 36-h duration of the hypercapnic conditions. Finally, air was readmitted and observations continued for up to 48-h post-hypercapnia.

Gas tensions in the aerial and aquatic compartments of the chamber were measured at various intervals throughout the experiment and mean values for specified time intervals are shown in table 1. Owing to the large size of the experimental chamber, the onset of hypercapnia was gradual with both compartments taking up to 1 h to reach an equilibrium of approximately 17 mm Hg. Ambient P_{CO_2} (Pw_{CO_2}) showed some tendency to increase as hypercapnia continued and by 24–36 h had reached 18–19 mm Hg. Such changes may have been bacterial in origin (*cf.* Mackenzie and Jackson, 1978). Although P_{O_2} tensions followed a reciprocal pattern, water oxygenation remained high during the experiments (table 1). The water in the tank was completely changed (flushed at same temperature and without apparent disturbance to the animal) 12 h before the experiment began and immediately following the period of hypercapnia.

MEASUREMENTS

Approximately 150 μl of blood was taken into a gas-tight Hamilton syringe for measurements of pH (Radiometer microelectrode) and total CO_2 (modified from Cameron, 1971). Replicate haematocrit tubes were filled directly from the cannula, immediately sealed and spun down. Samples of water or blood were analyzed for P_{O_2} and P_{CO_2} using Radiometer electrodes and display meters. Blood flow in the arterial catheter was sufficient to fill the electrodes in series and following determination, the blood was reinfused. Sampling caused no apparent disturbance to the animals. All electrodes were thermostatted to the experimental temperature of 25 °C with the exception of the total CO_2 electrode (37 °C). More detailed accounts of the analytical procedures have been given previously (McDonald *et al.*, 1980; Boutilier *et al.*, 1980).

In the interim between blood samples, arterial and buccal pressures were recorded by attaching the respective cannulae to Statham P23Db pressure transducers. The manometers were calibrated with static water columns at frequent intervals. The output from each transducer was recorded on a Beckman R511 Oscillograph.

Statistical analyses, when levels of significance are stated, were carried out using Student's '*t*' test.

Results

ACID–BASE RELATIONSHIPS IN ARTERIAL BLOOD

The relationships between all measured values of arterial blood pH, P_{CO_2} and total CO_2 (C_{CO_2}) during the time course of hypercapnia experiments on 7 *Cryptobranchus* are shown in fig. 1. Each mean (± SEM) corresponds only to those samples which fall within the time spans indicated by the shaded blocks (labelled A through E) on the abscissa (fig. 1).

The arterial blood acid–base status of resting undisturbed *Cryptobranchus* at 25 °C (Range A, fig. 1) are similar to previous data at the same temperature (Boutilier *et al.*, 1980). Plasma bicarbonate concentrations for Davenport analysis (fig. 2) were calculated from the measured values of pHa and Pa_{CO_2} with the Henderson-Hasselbalch equation ($\alpha CO_2 = 0.043$, $pk_1' = 6.17$; Boutilier and Toews, 1981) and gave similar results to measurements of $[HCO_3^-]$ in true plasma (Boutilier *et al.*, 1980).

The initial effects of hypercapnia (A–B, fig. 2) were gradual owing to the slow turnover time from airsaturated to hypercapnic conditions within the experimental chamber (table 1 and text). The decline in pHa and elevation of plasma $[HCO_3^-]$ represents a shift along a whole body buffer line whose slope ($\Delta HCO_3^-/\Delta pH$, N = 7) is −4.14 mmol/L · pH (A–B, fig. 2). The *in vivo* CO_2 titration (A–B) was complete for all animals by the 2nd h of hypercapnia and during the next 8 h, no consistent changes were detected (point B, figs. 1 and 2). During this initial equilibrium, the

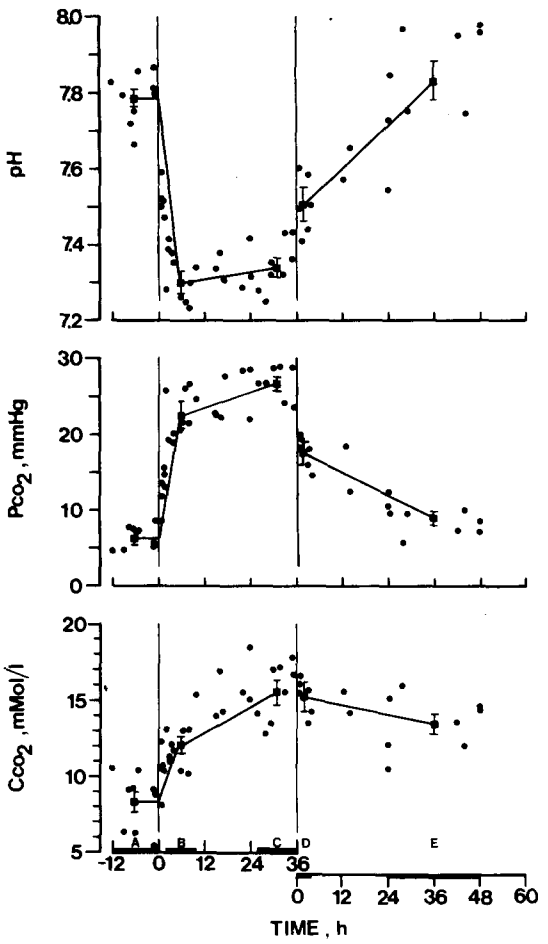

Fig. 1. Shown are the individual data points (●) for all measured values of arterial blood pH, P_{CO_2} and total CO_2 (C_{CO_2}) during hypercapnia experiments on seven *Cryptobranchus*. Mean values (± 1 SEM) represent periods of major steady state adjustment corresponding to samples taken within the time spans indicated by shaded blocks on abscissa (labelled A through E as described in text).

mean P_{CO_2} difference between arterial blood and ambient CO_2 tensions ($Pa_{CO_2} - PI_{CO_2}$) was not significantly different ($P < 0.01$) than that of animals in air-saturated conditions ($\Delta P_{CO_2} = 5.7 \pm 0.5$ mm Hg before CO_2 exposure, as against 5.5 ± 1.1 mm Hg during early hypercapnia; see table 1 for ambient CO_2 tensions).

The initial uncompensated respiratory acidosis was followed by a 16-h period in which plasma [HCO_3^-] increased along a gradually rising Pa_{CO_2} line (B–C) so that the net result was a slight pHa compensation (point C, figs. 1 and 2).

Upon abrupt re-exposure to air-saturated water (table 1), pHa increased and plasma [HCO_3^-] decreased along an *in vivo* buffer line (C through E, fig. 2) elevated above that seen during the initial response to hypercapnia (A–B) but having a much

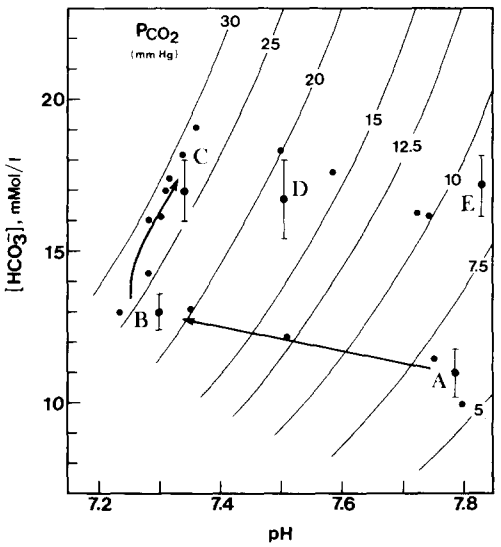

Fig. 2. Davenport diagram showing arterial blood plasma acid-base changes during hypercapnia experiments on seven *Cryptobranchus*. Mean (±1 SEM) plasma bicarbonate concentrations are plotted as a function of plasma pH for experimental stages A to E (time course as in fig. 1). Individual points represent data from one of the animals (850 g) contributing to the mean values shown. Arrows indicate the major movements.

TABLE 1

Gas partial pressures in the arterial blood (a) and in the aerial (I) and aquatic (w) compartments of experimental chamber during the course of hypercapnia experiments on seven *Cryptobranchus*. Values are means (±SE) of at least nine measurements at 25 °C

Experimental stage	P_{CO_2} (mm Hg)			P_{O_2} (mm Hg)		
	Pa_{CO_2}	PI_{CO_2}	Pw_{CO_2}	Pa_{O_2}	PI_{O_2}	Pw_{O_2}
Before hypercapnia	6.2 ± 1.2	≃0.5*	≃0.5*	27.4 ± 0.9	154.7 ± 1.4	153.4 ± 1.9
Early hypercapnia (2–10 h)	22.6 ± 1.8	17.4 ± 0.7	17.1 ± 0.4	34.2 ± 1.74	150.0 ± 2.0	148.5 ± 1.5
Late hypercapnia (26–36 h)	26.6 ± 0.74	18.3 ± 0.6	18.6 ± 0.7	31.1 ± 1.3	147.0 ± 2.4	145.5 ± 2.1
Post hypercapnia	17.5 ± 1.4	≃0.5*	≃0.5*	28.2 ± 3.5	155.1 ± 1.0	153.0 ± 2.0

* Not significantly different from measurements of room air or air equilibrated distilled water.

reduced mean slope (C–E $\Delta HCO_3^-/\Delta pH = -0.65$). One probable cause of the depression of the C–E slope is the overall reduction in haematocrit due to repetitive sampling (table 2), since a positive correlation between hct and blood buffering capacity is known to exist *in vitro* (Boutilier *et al.*, 1980).

TABLE 2

Changes in haematocrit (%) during hypercapnia experiments on seven *Cryptobranchus*. Values are means ± one standard error of mean. Stages A through E as described in text

	Experimental stage				
	A	B	C	D	E
Haematocrit (%)	28.1 (±2.3)	34.6* (±1.9)	29.8 (±2.4)	26.0 (±3.0)	20.7* (±3.8)

* Significantly different ($P < 0.001$) from pre-exposure stage A.

Noticeably, the early stage of the extracellular acid–base recovery was more rapid than later stages. Over the first 4 h (C–D) Pa_{CO_2} levels declined by approximately 10 mm Hg and then continued to fall, but over a progressively lengthening time course (D–E, figs. 1 and 2), taking up to 24 h or more to return to pre-exposure values. The recovery of pH floows a similar exponential like pattern, eventually resulting in pHa values higher than those seen in the untreated animal (E *vs.* A, figs. 1 and 2) ascribable to the new $[HCO_3^-]/[CO_2]$ equilibrium ratio (point E, figs. 1 and 2) which caused a metabolic alkalosis.

ARTERIAL BLOOD P_{O_2} AND % SATURATION ESTIMATES

The mean (± SEM) Pa_{O_2} of resting undisturbed *Cryptobranchus* is 27.4 ± 0.9 mm Hg (point A, fig. 3; table 1). Upon CO_2 exposure, Pa_{O_2} increased (A–B) and remained significantly elevated ($P < 0.01$) above pre-exposure levels for the 36-h duration of hypercapnia (B–C, fig. 3). In order to evaluate the physiological significance of these small Pa_{O_2} increases (5–10 mm Hg) when blood P_{CO_2} is elevated, we have constructed oxygen dissociation curves for the normal resting animal and for the hypercapnic animal (fig. 4) which are subject to the following limitations. The curve to the left, for the resting animal, is plotted in part from the mean values of five *in vitro* O_2 dissociation curves determined under the conditions of pH, P_{CO_2} and $[HCO_3^-]$ shown. The thickness of this illustrated curve also incorporates estimated values of P_{O_2} which are obtained when the Bohr factor is used to correct each P_{O_2} to a value (P_{O_2}') which corresponds to the mean *in vivo* pHa (pH′ 7.786) of resting animals in this study (*i.e.* pHa point A, figs. 1 and 2). This method was also used for constructing the curve to the right, based on 4 O_2 curves determined at an *in vitro* P_{CO_2} (22.1 mm Hg; Boutilier and Toews, 1981) which was close to the *in vivo* values at experimental stages B and C (figs. 1, 2 and 4). In using the formula, $\log (P_{O_2}'/P_{O_2}) = (\Delta \log P_{50}/\Delta pH) \cdot (pH'-pH)$, for values other than P_{50}, we have considered a small Bohr related increase in Hill's n to be negligible. For the purposes of the present analysis, the combined curves (*i.e.* measured plus estimated being

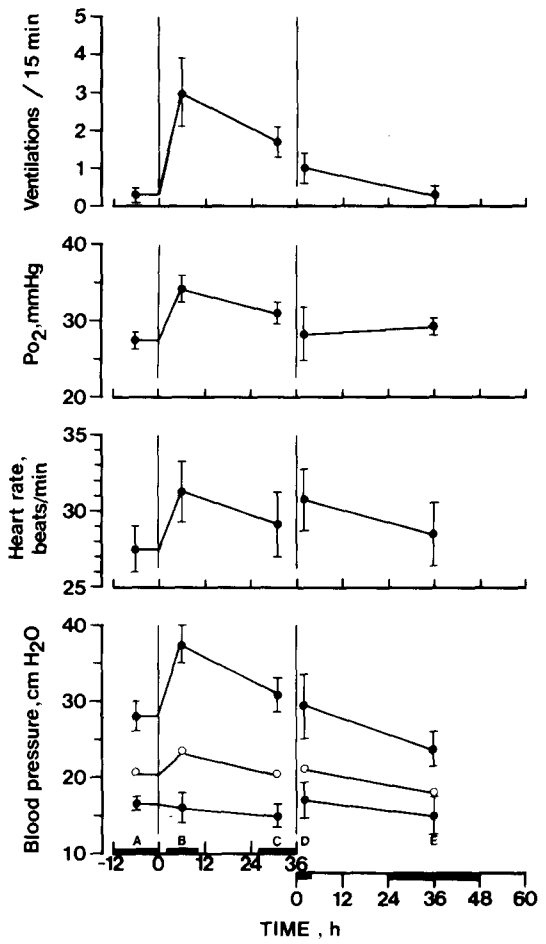

Fig. 3. Mean (± 1 SEM) values for lung ventilation frequency, arterial blood P_{O_2}, heart rate and blood pressure during hypercapnia experiments on seven *Cryptobranchus*. Time course and other criteria as in fig. 1. Blood pressure shown as mean systematic (O), systolic (above) and diastolic (below).

considered as approximate iso-pH and iso-P_{CO_2} lines), represent the normal resting condition (stage A) and that of hypercapnia in general (stages B and C, fig. 4). Direct plots of the mean Pa_{O_2} measurements corresponding to experimental stages A through C should therefore inform us of O_2–Hb saturation, but the analysis neglects possible blood O_2 content changes. Although hct increases at stage B (table 2), it is not clear whether this is attributable to erythrocyte swelling, due to the increased Pa_{CO_2} (see fig. 6 in Boutilier and Toews, 1981), or to haemoconcentration, since blood O_2 content measures are unfortunately lacking.

Certainly, if the rise in Pa_{O_2} during the initial stages of CO_2 exposure (A–B, fig. 3) did not occur, the overall effect would be a pronounced decline in arterial O_2 saturation to 45% (labelled A′, fig. 4). However, O_2 saturation in early hypercapnia

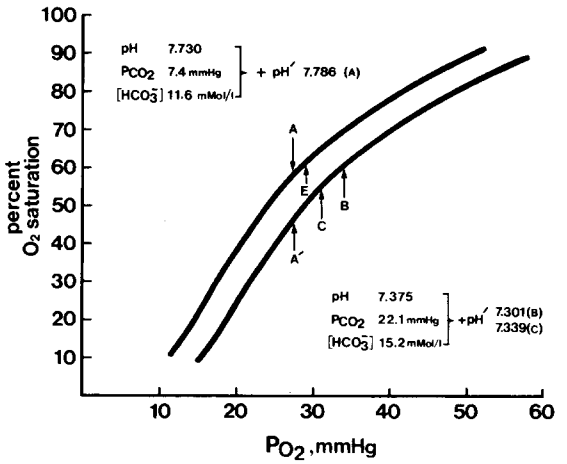

Fig. 4. O_2 dissociation curves representing *Cryptobranchus* whole blood at physiological levels of resting Pa_{CO_2} (7.4 mm Hg) and at Pa_{CO_2} levels seen during the hypercapnic period (22.1 mm Hg). Each curve is the sum total of measured O_2 curves under respective conditions of pH, P_{CO_2} and $[HCO_3^-]$ shown, together with curves reconstructed using Bohr factor to adjust P_{O_2} levels to *in vivo* pHa (pH′) at experimental stages A–C in present study. Mean values (N = 7) for *in vivo* arterial blood P_{O_2} are shown for stages A–C and E. Refer to text for explanation of A′ and other details.

(point B) is maintained at or near that seen in the resting animal (point A, fig. 4). More prolonged CO_2 exposure (point C) results in a small but nonsignificant ($P > 0.05$) decline to a mean O_2 saturation of 53%. PH based estimates of arterial O_2 saturation in the latter recovery stages (point E, figs. 1–4) show that the blood O_2 relationships had essentially returned to normal.

VENTILATION

Cryptobranchus in air-saturated conditions (25 °C) ventilates its lungs in a highly infrequent manner (Boutilier *et al.*, 1980), ranging from zero to 3 per hour in the present study (stage A, fig. 3). Selected recordings of buccal pressure measurements taken during various stages of the hypercapnic period (B–C) are shown in fig. 5. More often than not, similar 5-min periods of buccal readings from normocapnic animals in air-saturated conditions revealed no buccal activity. However, lung ventilations, whether in normocapnic or hypercapnic animals were usually of the pattern seen in fig. 5. In both conditions, excursions to the surface were generally short lived (10–15 s), resulting in a single lung ventilation recorded in the buccal cavity as several buccal oscillations which precede a large positive pressure deflection when air is forced into the lungs. Following inspiration, the animal immediately submerges and within 5–10 s expels a bolus of air as bubbles through the mouth and/or spiracular openings (fig. 5b).

In the early and later stages of hypercapnia, ventilations were often followed

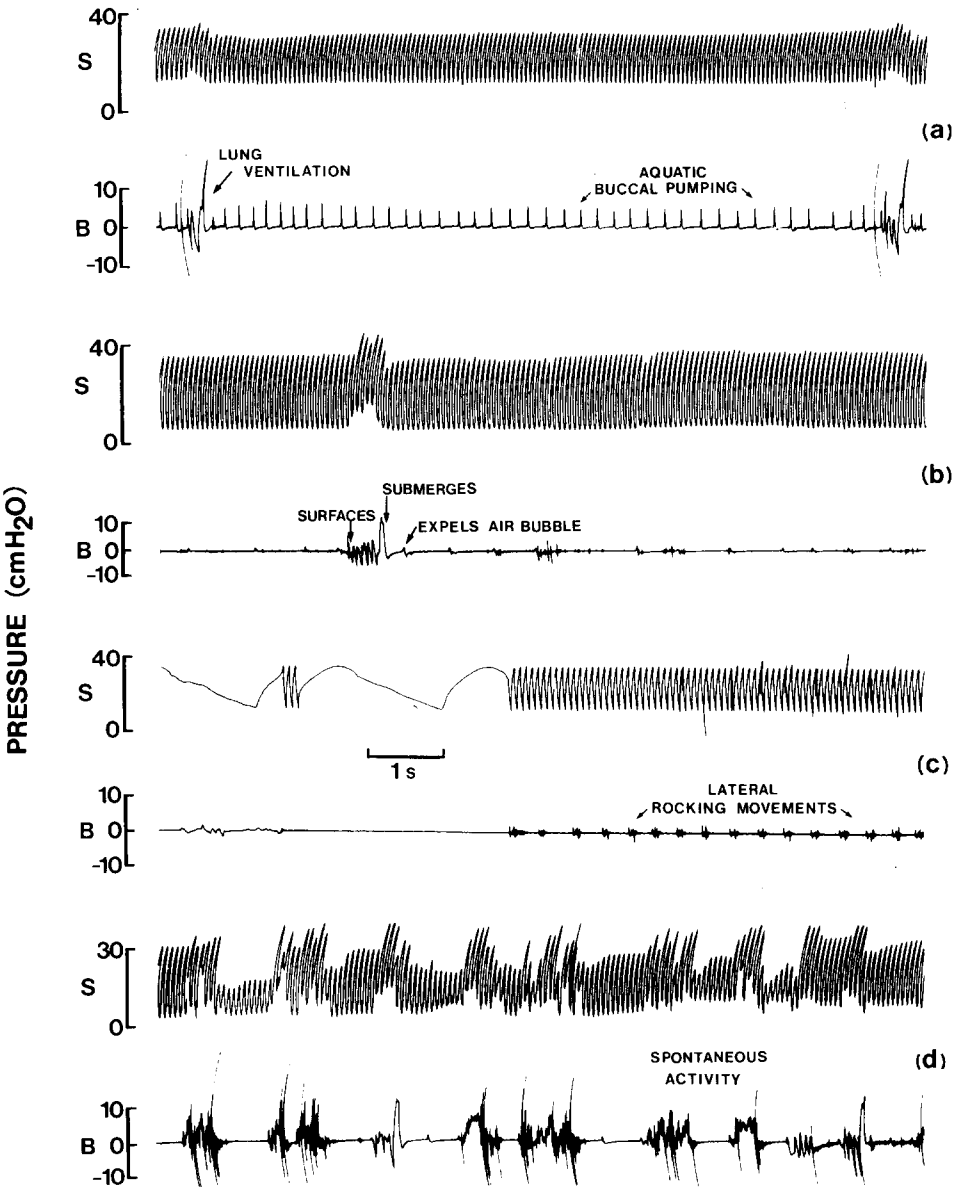

Fig. 5. Selected recordings of buccal cavity (B) and systemic (S) pressures during the hypercapnic exposure period in *Cryptobranchus*. Records are not intended as representative of any one stage of hypercapnia but rather to illustrate some of the patterns which were observed most often over the course of the present experiments.

with underwater buccal movements which vary from being high pressure and regular (fig. 5a) to low pressure and relatively infrequent (fig. 5b), the latter of which were sometimes recorded in normocapnic animals. Submerged *Cryptobranchus* also exhibits a characteristic body movement whereby the animal rocks or sways in a lateral fashion. These side to side motions, which are presumed to be a rudimentary form of convection (Guimond and Hutchison, 1973; Boutilier and Toews, 1981) were often fortuitously recorded as discrete bursts of noise (*i.e.* fig. 5c) generated by the corresponding movements of the pressure cannulae. While exposure to elevated levels of ambient P_{CO_2} brought about increases in all respiratory activities (fig. 5), the extent to which the underwater components (buccopharyngeal pumping and rocking movements) contribute to aquatic gas exchange are not yet known. However, their increased frequency during hypercapnia and hypoxia (unpublished observations) suggests that they may be an important adjunct to cutaneous gas exchange.

From the work of Guimond and Hutchison (1973), we do know that the lungs alone may account for 10% $\dot{V}_{O_2}$ and less than 3% $\dot{V}_{CO_2}$ of resting *Cryptobranchus* at 25 °C. Lung ventilation frequency increased during early CO_2 exposure and remained significantly elevated ($P < 0.01$) for the duration of the hypercapnic period (B–C, fig. 3). Most hypercapnic animals exhibited some degree of spontaneous activity (for up to 15 min) and these sessions invariably contained a concentrated bout of aerial gas exchanges (fig. 5d). During the early stages of recovery all respiratory activities declined but not until the latter periods were they within the levels of variability seen in pre-exposure animals.

CIRCULATION

The mean systemic blood pressure of resting animals (point A, fig. 3) is similar to that which we reported earlier (Boutilier *et al.*, 1980). However, the corresponding heart rate of animals in this study is on average about 15 beats/min lower than the previous determinations at the same temperature and season. The only difference of any note between this and the previous work was the design and size of the experimental chamber.

During all phases of the experiments, heart rate and mean systemic blood pressure exhibited a high degree of individual variation. After the initial exposure to CO_2, the heart rate and blood pressure of most animals increased by a small margin (A–B, fig. 3) but thereafter, no consistent patterns could be seen. The increases in pulse pressure were usually caused by a large increase in systolic pressure although it should be mentioned that diastolic pressures often declined by a far greater extent than is apparent in fig. 3. At early (D) and later (E) stages of recovery, the circulatory parameters were not significantly different ($P > 0.10$) from the pre-exposure controls (A, fig. 3). Recorded examples of the pulse pressures of four hypercapnic animals are shown along with the corresponding respiratory activities in fig. 5.

Discussion

RESPIRATORY CONTRIBUTIONS TO ARTERIAL BLOOD GASES

When *Cryptobranchus* is exposed to elevated levels of environmental CO_2, it appears at first that little if any of the whole body buffering can be attributed to enhanced CO_2 losses at the respiratory surfaces; *i.e.* the P_{CO_2} difference between arterial blood and ambient media (Pa_{CO_2}–PI_{CO_2} remains the same whether the salamander is in air-saturated (point A) or hypercapnic (point B) conditions (table 1; figs. 1 and 2). This initial interpretation would, however, rest on the hypothesis of a purely diffusion limited cutaneous CO_2 exchange (*i.e.* Piiper *et al.*, 1976; Jackson, 1978) and of a constancy of the metabolic rate. It seems possible that some degree of accelerated CO_2 elimination could be offered by the increased respiratory activities during hypercapnia (lung ventilation, aquatic buccopharyngeal pumping, whole body convection; figs. 3 and 5) since their presence indicates a direct CO_2 sensitive respiratory response. Such could be the case if for example aerobic metabolism was elevated by the CO_2 exposure. Perfusion adjustments could also contribute to the efficiency with which gas is transferred since the large increase in systemic pulse pressure during hypercapnia (fig. 3) is some indication that blood flow is augmented in the peripheral vasculature. Even so, the potential for increasing gas exchange through selective perfusion adjustments must be functionally reduced in *Cryptobranchus*, relative to other amphibians (reviewed in Shelton, 1976), by the lack of separate oxygenated and deoxygenated bloodstreams leaving the heart (Guimond and Hutchison, 1973; Boutilier and Toews, 1981). An indication of the relative efficiency with which Pa_{CO_2} is adjusted, by active ventilatory or other means, can be afforted by comparing the respiratory acid-base responses of the hellbender with those produced by the controlled pulmonary ventilation of predominantly air breathing species.

In previous experiments on the semi-terrestrial anuran, *Bufo marinus*, it was found that an abrupt increase in PI_{CO_2} led to a marked and immediate hyperventilatory response (Macintyre and Toews, 1976) which had the effect of regulating respiratory acid–base balance through a considerable reduction of the pre-exposure Pa_{CO_2}–PI_{CO_2} (*i.e.* 12 mm Hg at rest as against 2 mm Hg in 5% CO_2; Boutilier *et al.*, 1979a). It can be seen from fig. 2 that *Cryptobranchus*, with a resting Pa_{CO_2}–PI_{CO_2} of approximately 6 mm Hg (point A) and an *in vivo* buffer slope of −4.14 (A–B), would have benefited quite extensively, in terms of pHa compensation, through a respiratory (ventilatory) adjustment of similar proportion to that observed in the toad. It seems clear that the degree of Pa_{CO_2} regulation offered by the sum total (principally skin) of respiratory responses in *Cryptobranchus* is much less precise than that afforded by the pattern and frequency of lung ventilation in semi-terrestrial anurans. These differences are certainly explicable on the grounds that amphibian gas exchange may be largely passive (*i.e.* constant skin CO_2 conductance; Jackson and co-workers) however, it does not explain the increased presence of active breathing movements in response to an elevation of PI_{CO_2} (fig. 5).

It seems more likely from our results that the auxiliary respiratory activities are primarily directed toward the supply of oxygen rather than the elimination of CO_2. The O_2 curve analysis in fig. 4 shows that, if not for the increases in Pa_{O_2} during the CO_2 exposure period (fig. 3), the ensuing respiratory acidosis (A–B, figs. 1 and 2) would have resulted in a marked CO_2-mediated arterial hypoxaemia (point A′, fig. 4). If the rise in Pa_{O_2} is caused by enhanced pulmonary (or other) ventilation in hypercapnia, it is difficult to understand why Pa_{CO_2}–PI_{CO_2} goes unchanged (table 1) unless there is, for example, an overall change in aerobic metabolism. Though the absolute increases in Pa_{O_2} are small, their physiological importance can be realized in that arterial O_2 saturation is preserved within narrow limits.

EXTRACELLULAR ACID–BASE ADJUSTMENTS

During the early stages of whole body CO_2 titration, a respiratory acidosis developed along an *in vivo* true plasma slope (A–B, fig. 2) which was considerably reduced from that obtained on blood samples equilibrated *in vitro* (Boutilier and Toews, 1981). These differences in slope reflect the buffering which takes place *in vivo* when plasma bicarbonate, formed by the non-carbonic blood buffering system, is redistributed (*i.e.* net efflux) throughout the total extracellular and various other body fluid compartments. In the absence of any apparent ventilatory contribution to reduce the Pa_{CO_2}–PI_{CO_2} gradient, the initial respiratory acidosis (point B, fig. 2) appears to be totally uncompensated for the first 10 h of CO_2 exposure. Presumably, the subsequent and gradual accumulation of plasma bicarbonate (B–C, fig. 2) reflects an involvement of slower acting buffering processes such as renal compensation or ionic exchange mechanisms at the skin, although their probable contributions to whole animal acid–base maintenance have yet to be explored. What is evident is that the compensatory gain in plasma [HCO_3^-] had little overall effect in the restoration of extracellular pH (B–C, fig. 2) and one wonders what physiological advantage, unless towards the preservation of pHi (Toews and Heisler, 1980), is achieved by this secondary phase of acid-base adjustment.

The recovery period studied consists of a prolonged respiratory adjustment whereby the animal is slowly backtitrated along an elevated *in vivo* buffer line (C through E, fig. 2). Although pHa and Pa_{CO_2} are eventually restored, there is no indication that the bicarbonate gained during the compensatory period (B–C, fig. 2) is being redistributed.

RESPIRATORY CO_2 ADJUSTMENTS DURING RECOVERY

The respiratory adjustment (*i.e.* decline in Pa_{CO_2} which brings about the restoration of pHa can be observed to follow an exponential time course; an initial rapid fall in Pa_{CO_2} (30–60 min, C–D) followed by a progressively lengthening decay

(D–E, fig. 1). If we consider that the abrupt return to air saturated water effectively sets up a transcutaneous Pa_{CO_2}–PI_{CO_2} gradient in the order of 25 mm Hg (time zero recovery, fig. 1) and that respiratory CO_2 losses are primarily diffusion limited, an exponential decline in Pa_{CO_2} would be predicted. A similar pattern was observed when Pa_{CO_2} levels in *Cryptobranchus* were gradually restored to normal after a rise in tissue CO_2 output (Boutilier *et al.*, 1980). Moreover, it is significant that the analysis of inert gas elimination from the essentially skin breathing Plethodont, *Desmognathus fuscus*, also reveals an exponential pattern of decay (Gatz *et al.*, 1975; Piiper *et al.*, 1976). Finally, a recent study of acid–base balance and temperature in *Cryptobranchus* indicates that skin CO_2 loss can be significantly accelerated only by increasing Pa_{CO_2} whether the animals have access to air or not (Moalli *et al.*, 1981). Taken together, the above observations suggest that respiratory CO_2 losses in *Cryptobranchus* are either passive or at best only poorly controlled.

In conclusion, the results on acid-base balance and ventilation in hypercapnic exposed *Cryptobranchus* appear analogous with the situation described for teleost and elasmobranchid fish (Randall *et al.*, 1976; Janssen and Randall, 1975, Cameron, 1978). In both cases, a rise in PI_{CO_2} leads to increased ventilatory activity which is not explicable in terms of respiratory acid–base maintenance; Pa_{CO_2} rises by the same amount as PI_{CO_2}. Unlike mammals and other lung breathing vertebrates these aquatic animals are unable, through ventilatory or other means, to adjust the Pa_{CO_2}–PI_{CO_2} so as to enhance CO_2 losses and thereby partially compensate the ensuing respiratory acidosis. Instead, the compensatory pHa adjustments which do occur are effected through longer term (nonventilatory) buffering processes which bring about increased amounts of plasma bicarbonate. In the absence of any clear cut respiratory CO_2 control in *Cryptobranchus*, the apparent regulation of arterial blood oxygenation (figs. 3 and 4) leads one to suspect that the increases in ventilatory activities are activated by an O_2-sensitive mechanism, mediated by the CO_2 dependent Bohr shift. A response of this sort would be more in line with thereoretical arguments regarding the gas exchange requirements of other predominantly water breathing vertebrates (Rahn, 1966; Dejours, 1975).

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