

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*

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SUMMARY

A combined respiratory and metabolic acidosis occurs in the arterial blood immediately following 30 min of strenuous activity in the predominantly skin-breathing urodele, *Cryptobranchus alleganiensis*, and in the bimodal-breathing anuran, *Bufo marinus*, at 25 °C. In *Bufo*, the bulk of the post-exercise acidosis is metabolic in origin (principally lactic acid) and recovery is complete within 4-8 h. In the salamander, a lower magnitude, longer duration, metabolic acid component and a more pronounced respiratory acidosis prolong the recovery period for up to 22 h post-exercise. It is suggested that fundamental differences between the dominant sites for gas exchange (pulmonary versus cutaneous), and thus in the control of respiratory acid-base balance, may underline the dissimilar patterns of recovery from exercise in these two species.

INTRODUCTION

Although much is known about the energetics of activity metabolism in amphibians (reviewed by Bennett, 1978), there is practically no information about cardio-ventilatory adjustments or changes in blood acid-base balance associated with exercise in these animals. In the preceding paper (McDonald, Boutilier & Toews, 1980), we have examined some of the latter responses to exercise in the semi-terrestrial anuran, *Bufo marinus*, and found that respiratory and non-respiratory acids are retained in disproportionate amounts in the arterial blood so that 90% of the post-exercise acidosis is metabolic in origin. This toad possesses a highly elaborate alveolar architecture (Smith & Campbell, 1976) and can, through pulmonary ventilation, effectively control the respiratory component of blood acid-base balance (Boutilier *et al.* 1979*a, b*).

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Whilst the partitioning of anuran gas exchange between lungs and skin is historically well documented (Krogh, 1904; Dolk & Postma, 1927; Vinegar & Hutchison, 1965; Hutchison, Whitford & Kohl, 1968; Shelton, 1976), it has been only recently that the control of this partitioning was found to reside with pulmonary ventilation, at least in the bullfrog, *Rana catesbeiana* (Gottlieb & Jackson, 1976; MacKenzie & Jackson, 1978; Jackson, 1978) and probably for the toad, *Bufo marinus* (Boutilier *et al.* 1979 *a, b*). It is this ventilatory control which, in the active toad, appears to play the key role in minimizing the rise in arterial blood  $P_{CO_2}$  and, in so doing, avoids the possible cumulative effects that a large respiratory and metabolic acidosis would have on arterial blood pH.

Respiratory gas exchange mechanisms are only poorly understood in those amphibians which respire predominantly through the skin but recent evidence (Gatz, Crawford & Piiper, 1975; Piiper, Gatz & Crawford, 1976; Boutilier, 1978) suggests that, relative to the lung-breathing Anura, a precise control of arterial blood  $P_{CO_2}$  may be more difficult to achieve. If so, we might expect the blood acid-base disturbance resulting from strenuous activity in skin-breathers to have a more pronounced respiratory acid component than was observed in our previous study on the lung-breathing toad, *Bufo marinus* (McDonald *et al.* 1980). Thus, in the present study we chose to examine ventilation, circulation and blood acid-base correlates of activity metabolism in a predominantly cutaneous-breathing urodele, *Cryptobranchus alleganiensis*. Adult *Cryptobranchus* lacks gills and its lungs are poorly vascularized, non-septate sacs which contribute little to overall gas exchange (Guimond & Hutchison, 1973, 1976). Its skin, however, is richly vascularized with capillaries which penetrate into the surface cell layer of the epidermis. An increased surface area to volume ratio is provided by a dorso-ventrally flattened form with reticulated folds of skin hanging from the margins of the body and legs (Noble, 1925).

#### MATERIALS AND METHODS

##### *Experimental animals*

*Cryptobranchus alleganiensis alleganiensis* (Daudin), ranging in weight from 300 to 700 g, were collected in Missouri river drainages and air-shipped to Nova Scotia soon after capture. In the laboratory the animals were kept in a large, well-aerated water tank ( $25 \pm 2$  °C) and were apparently healthy at the time of experimentation.

##### *Animal preparation*

Arterial and buccal catheters were chronically implanted after the salamanders had been anaesthetized by immersion in a 0.035% solution of neutralized MS-222 (pH of the acid salt adjusted to 7.6–7.8 as suggested by Ohr, 1976 *a, b*). At this concentration and pH, anaesthesia usually occurred within 15–20 min and the animals remained anaesthetized for at least 1 h after being removed from the solution. During surgery (20–30 min), the animals were placed in a shallow pan of water and any exposed skin was kept moist with wet cloths.

The site chosen for arterial cannulation was the conus arteriosus, which leaves the ventricle as a large single vessel and, within a distance of 10–15 mm, expands to

form the bulbous arteriosus and associated aortic arches. Preliminary attempts to cannulate more distal regions (i.e. mesenteric arteries or dorsal aorta) were found to be unsatisfactory (Boutillier, 1978) due to the profuse bleeding caused by necessarily large approach incisions through the richly vascularized belly skin and underlying musculature. In our hands, the heart offered the least disruptive site. Access to the conus was gained by a 3–4 cm incision through the ventral skin overlying the region of the heartbeat. Cutting through a small portion of the cartilaginous region of the pectoral girdle was unavoidable in retracting the sternohyoideus muscle and exposing the pericardial cavity. A 1–2 cm incision was made in the pericardium and the conus was gently lifted so that a section of it could be closed off with fine artery clamps. A small hole was then made in the arterial wall with a 26-gauge hypodermic needle and a catheter was inserted through this hole in an upstream direction so that 7–10 mm of the tubing lay within the vessel. The catheter was anchored in position at the entry point by looping a piece of fine surgical silk around the tubing so as to trap a continuous piece of the surrounding connective tissue on the outside of the vessel wall. This method always ensured a tight seal and blood loss was invariably negligible. Depending on the size of the conus, arterial catheters were fashioned from either P.E. 50 (0.58 mm bore, 0.97 mm diam.) or P.E. 60 (0.76 mm bore, 1.22 mm diam.) polyethylene tubing and in no instance did it seem likely that blood flow was occluded to a significant extent. Prior to insertion, all catheters were filled with heparinized (200 i.u./ml) Mackenzie saline (de la Lande, Tyler & Pridmore, 1962) to prevent clotting and 0.1 ml/kg of this solution was injected immediately following placement of the cannulae. Catheters were secured to the surrounding musculature with surgical silk and all incisions were carefully closed with interrupted sutures.

Fluid-filled catheters were implanted into the buccal cavity by making a hole through the cartilaginous portion of the upper jaw with a 15-gauge hypodermic needle and threading a length of P.E. 50 polyethylene tubing through its bore. The implanted end was then heat flared and drawn back firmly to form a tight fit with the roof of the mouth.

#### *Experimental protocol*

Following the operation, the animals were allowed 24 h in which to recover and acclimate to the experimental chamber, a temperature-controlled ( $25 \pm 0.5$  °C) 30 l water bath. The bath was vigorously aerated with several airstones and flushed with fresh water (same temperature and without any apparent disturbance to the animal) at periodic intervals prior to and thereafter throughout the duration of the experiment.

In preliminary tests on unoperated salamanders, attempts to produce continuous activity by mechanical means, such as those used for the toad (McDonald *et al.* 1980), proved unsuccessful. The animals could, however, be induced to swim nearly continuously by delivering periodic and small voltage shocks to the tail with hand-held electrodes, and were chased about the tank in this fashion for 30 min. Eliciting activity by prodding alone, without the use of shocks, was favoured but not always possible. The quantification and repeatability of an exercise protocol, such as the water-tunnel method used for the salamander, *Ambystoma tigrinum* (Cushman, Packard & Boardman, 1976), is clearly desirable, but was not applicable in the case of *Cryptobranchus*.

At the start of each experiment, an arterial blood sample and a 2 h record of blood and buccal pressures were obtained from the resting animal. Blood samples were subsequently taken immediately following activity (time 0) and at +30 min, 1 h, 2 h, 6 h, 10 h and 22 h post-exercise. Between blood samples, recordings of buccal and blood pressure were continuous except for the 10–22 h period.

### *Analytical procedures*

For each blood sample, the pressure in the arterial catheter was always sufficient to rapidly fill a 250  $\mu\text{l}$  Hamilton syringe barrel and two haematocrit tubes. The latter were immediately sealed with wax and spun down. If the gas tight plunger was inserted so as just to make a seal, this provided a 300  $\mu\text{l}$  sample which was sufficient for determinations of pH, total  $\text{CO}_2$  ( $C_{\text{CO}_2}$ ) and L-lactate as detailed earlier (McDonald *et al.* 1980). The  $C_{\text{CO}_2}$  of true plasma was measured for the resting sample only and the calculation of plasma bicarbonate concentrations by this method tallied ( $P < 0.01$ , Student's paired  $t$ -test) with those estimated from the Henderson–Hasselbalch equation (see below for  $\alpha\text{CO}_2$  and  $\text{pK}'_1$  values) using pH and  $P_{\text{CO}_2}$  measurements. The pH measurements of true plasma were equally reliable (99% confidence) with our blood measurements indicating that the centrifugation for true plasma was done under strictly anaerobic conditions (see McDonald *et al.* 1980). This check on true plasma served to validate the measured variables, our particular concern being with the reliability of measuring  $P_{\text{CO}_2}$  at the relatively low absolute levels (5–7 mmHg) encountered in the arterial blood of *Cryptobranchus*.

Arterial  $\text{CO}_2$  tension ( $P_{a,\text{CO}_2}$ ) was measured immediately following the blood collection, by allowing the pressure in the catheter to twice fill the electrode chamber; the methodology is described elsewhere (Boutilier *et al.* 1978, 1979a; McDonald *et al.* 1980). Blood used for this measurement was thereafter returned to the animal.

Blood pressure and breathing rates were recorded on a Beckman R511 oscillograph by connecting the respective cannulae to Statham pressure transducers (Type P<sub>23</sub>Db). These manometers were calibrated before and at frequent intervals throughout the experiment.

### *Calculations*

Bicarbonate concentrations in whole blood and true plasma were calculated using the formula:

$$[\text{HCO}_3^-] = \text{Ca}_{\text{CO}_2} - \alpha\text{CO}_2 \cdot P_{a,\text{CO}_2}, \quad (1)$$

where  $\text{Ca}_{\text{CO}_2}$  and  $P_{a,\text{CO}_2}$  are measured quantities and  $\alpha\text{CO}_2$  (0.043 m-mol  $\text{CO}_2 \cdot \text{l}^{-1} \cdot \text{mmHg}^{-1}$ ) is an experimentally derived  $\text{CO}_2$  solubility coefficient for *Cryptobranchus* plasma at 25 °C (Boutilier, 1978). The calculations of plasma  $[\text{HCO}_3^-]$  by the Henderson–Hasselbalch equation, as indicated earlier, utilized a plasma  $\text{pK}'_1$  value of 6.17 (at 25 °C) which was determined gasometrically by Boutilier (1978).

Post-exercise levels of buffered respiratory acid ( $\text{H}_2\text{CO}_3$ ) were estimated by graphical interpretation of the Davenport diagram (Woodbury, 1974; Wood, McMahon & McDonald, 1977). The changes in buffered metabolic (non-volatile) acid levels were calculated by the equation given in the preceding paper (McDonald *et al.* 1980). For this equation, the value  $\beta$  (the slope of the non-bicarbonate buffer

line,  $\Delta[\text{HCO}_3^-]/\Delta\text{pH}$ ) was obtained from the linear relationship found between haematocrit (at resting  $P_{a,\text{CO}_2}$ ) and buffering capacity for *Cryptobranchus* blood at 25 °C (Boutilier, 1978). This relationship, described by the regression equation

$$\beta = -0.48 \text{ hct} - 1.98 \quad (2)$$

$$(r = 0.965, \text{ S.E. slope} = 0.02, \text{ S.E. intercept} = 0.51)$$

was consistent ( $P < 0.01$ ) with the values empirically determined *in vitro* on four of the animals used in this study. In all instances, the slope  $\beta$  was estimated from the above equation using the haematocrit value determined prior to exercise.

## RESULTS

Cardioventilatory and blood acid-base correlates of activity metabolism in *Cryptobranchus* will be examined and discussed in comparison to the applicable data reported for the toad, *Bufo marinus*, in the preceding paper (McDonald *et al.* 1980). Data for both species were collected in a similar fashion and at the same temperature, the major technical divergence being the way in which activity was enforced (see Methods). Pilot experiments on three *Cryptobranchus* indicated that 30 min of continuous swimming movements resulted in an arterial blood acidosis (assessed by  $\text{pH}_a$ ) similar in magnitude to that provoked by the exercise protocol used for the experiments on the toad. While using this similarity as the criteria for comparison we do not, however, suggest that it may necessarily reflect a comparable quantitative measure of energy expenditure. Nevertheless, the intra- and interspecific levels of the arterial blood acidosis were surprisingly consistent considering the qualitative measures of activity that were employed in both instances. Furthermore, all animals of both species fully recovered from the activity procedures and thereafter remained in a healthy state.

Results of the cardioventilatory responses of *Bufo* to 30 min of activity are given by McDonald *et al.* (1980) and will be referred to in the Discussion only. The blood acid-base correlates of exercise for both animals are, however, graphically presented (Figs. 2 and 3) for purposes of comparison.

### Ventilation

Lung ventilatory frequencies of resting *Cryptobranchus* in air-saturated water were always infrequent (Fig. 1A) ranging from zero to six per hour in the present study ( $N = 8$ ). Similar observations indicate that periods of 1–2 h may elapse between excursions to the surface to breathe (Boutilier, 1978). Inspiration is usually terminated after 2–3 buccal movements, the last of which is usually a large swallowing action whereby air is forced into the lungs. Upon submergence, a surplus of air is removed as bubbles through the spiracular openings and the animal gradually assumes its resting posture.

In addition to these periodic lung ventilations are at least two underwater activities which may also play a role in gas exchange. On occasion, normal periods of motionless rest at the bottom of the aquaria are punctuated with body movements whereby the animal rocks or sways in a lateral fashion. This behaviour causes the skin folds to

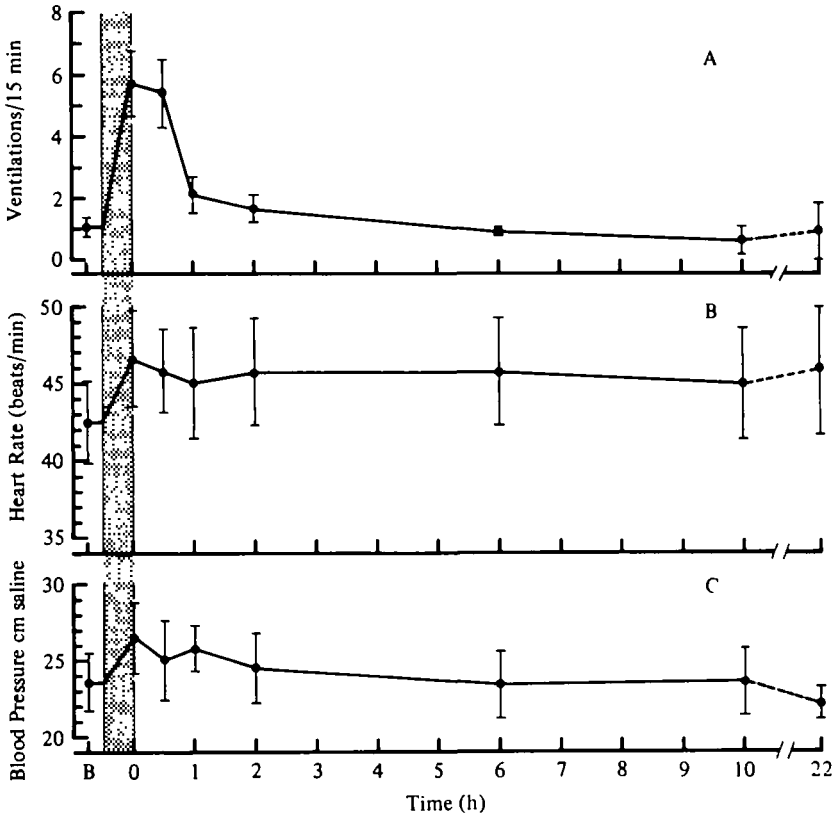


Fig. 1. (A) lung ventilation frequency, (B) heart rate and (C) mean arterial blood pressure for *Cryptobranchus alleganiensis* (means  $\pm$  1 S.E.M.,  $N = 8$ ) before and after 30 min of enforced activity. Shaded area = exercise period. Time B = initial resting sample. Time 0 = immediately post-exercise. Temp. = 25 °C.

undulate and may assist in disturbing the boundary layer between ambient water and skin. Usually associated with these rocking movements are small pressure deflexions of the buccal floor which appear to flush the oropharyngeal cavity with water. These activities occur more regularly when the ambient medium becomes hypoxic or hypercapnic and a more detailed account is forthcoming (R. G. Boutilier and D. P. Toews, in preparation). Although not quantified in this investigation, it was evident both from our observations and chart recordings, that the rocking and underwater buccal movements increased during and following exercise even though the ambient water was continuously air-saturated.

Thirty minutes of enforced activity resulted in a significant increase in the frequency of lung ventilations (Fig. 1 A); the extreme of which in one animal was 36/h. It should be noted, however, that these elevated breathing frequencies following exercise were not accompanied by an equal number of excursions to the surface. Instead, the majority of the ventilations were contributed during sustained bouts of breathing movements during three or four surfacing episodes of uncharacteristic duration, the remainder of which are attributable to the discrete ventilations mentioned above. By 1 h post-exercise these patterns subsided and the ventilatory rates and patterns were indistinguishable from those seen in animals at rest.

### Circulation

The post-exercise changes in heart rate (Fig. 1 B) and mean systemic blood pressure (Fig. 1 C) were extremely modest and showed a considerable amount of individual variability. These factors preclude any meaningful statistical comparisons of the mean values, and examination of variables such as body weight failed to show the emergence of any clear patterns.

#### Acid-base relationships in arterial blood

##### 1. Pre-activity

At 25 °C, the resting  $P_{a,CO_2}$  in *Cryptobranchus* was  $5.9 \pm 0.4$  mmHg ( $\bar{x} \pm 1$  S.E.M.,  $N = 8$ , Fig. 2 C). This value is approximately one-half that seen in the arterial blood of *Bufo* (Fig. 2 C). Despite these large differences in resting  $P_{a,CO_2}$ , both species maintain about the same  $pH_a$  (Fig. 2 D) with the linear/log relationship between  $P_{CO_2}$  and  $pH$  being matched by an appropriate concentration of blood bicarbonate (Fig. 2 B). These resting values for both animals (Fig. 2 B–D) are in good agreement with data which were independently collected from chronically catheterized *Cryptobranchus* (Boutilier, 1978) and *Bufo marinus* (Howell *et al.* 1970; Reeves, 1977; Boutilier *et al.* 1979a) at 25 °C.

Lactate concentrations in the arterial blood of undisturbed *Cryptobranchus* ( $0.56 \pm 0.10$  m-equiv.l<sup>-1</sup>) and *Bufo* ( $0.41 \pm 0.05$  m-equiv.l<sup>-1</sup>) were found to be uniformly low (Fig. 2 A). These values are substantially below most previously reported levels for a wide variety of amphibians (see review by Bennett, 1978). The disparity is very likely methodological in origin, reflecting the assortment of techniques which have been used for blood collection. When blood samples are withdrawn from a catheter which has been chronically implanted, and animals are then given a suitably long period to recover from the surgery, the levels of arterial blood lactate are invariably low for many ectothermic species (Gottlieb & Jackson, 1976; Driedzic & Kiceniuk, 1976; Wood *et al.* 1977; D'Eon, Boutilier & Toews, 1978; McDonald, McMahan & Wood, 1979). These latter studies further emphasize the importance of chronic cannulation methods for determining blood acid-base variables from animals in steady-state conditions.

##### 2. Post-activity

In both species, the depression in  $pH_a$  was greatest immediately following exercise (time 0, Fig. 2 D) and this was also the time when peak concentrations of lactate appeared in their arterial bloodstreams (Fig. 2 A). In *Cryptobranchus*, however, the levels of lactate attained immediately following activity stayed rather constant over the subsequent 2 h of recovery and then only slowly began to approach (+ 10 h) and eventually reach (10–22 h) the pre-exercise concentrations. In contrast, the disappearance of blood lactate in *Bufo* was comparatively rapid and the exponential pattern of its descent (half-time of 2 h) closely mirrors the recovery patterns of arterial blood  $[HCO_3^-]$  and  $pH$  (Fig. 2).

Increases in  $P_{a,CO_2}$  accompany the exercise periods of both animals but in *Cryptobranchus* the rise is initially twofold greater than in *Bufo* (time 0, Fig. 2 C). Furthermore, the toad rapidly restores its  $P_{a,CO_2}$  (+ 30 min), whereas in *Cryptobranchus* a

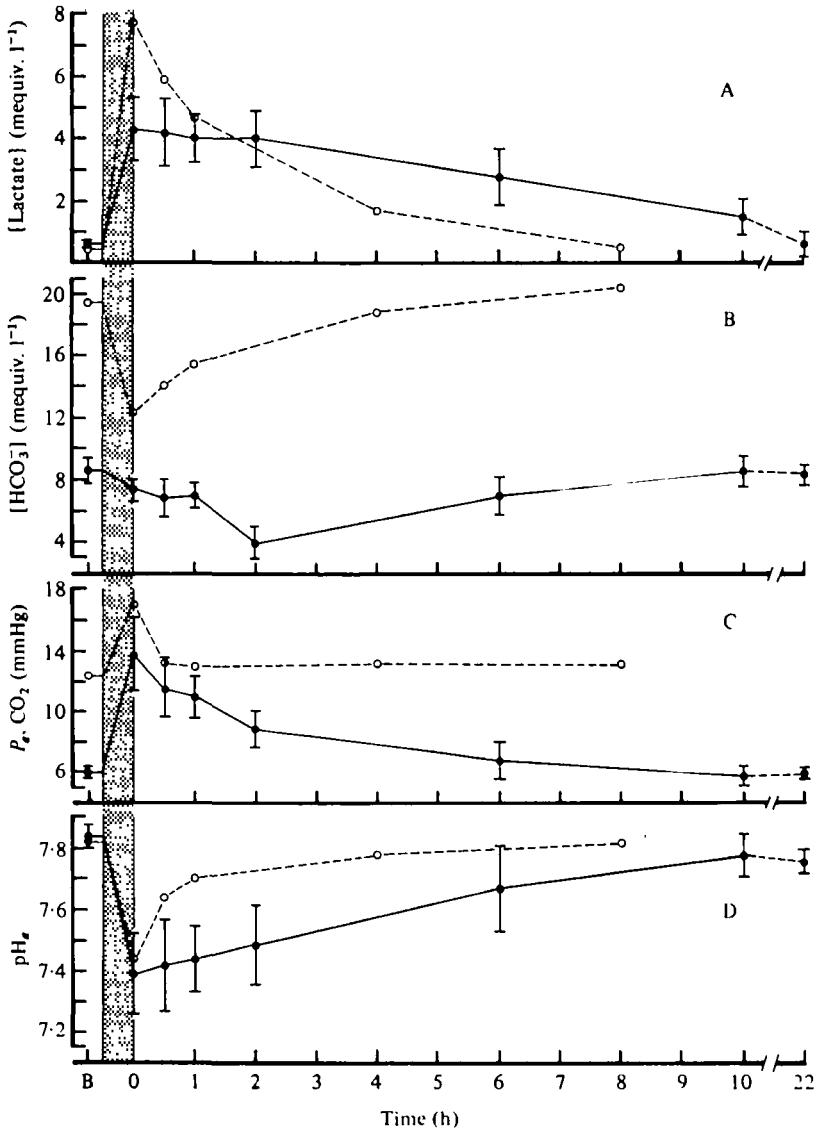


Fig. 2. (A) lactate, (B) bicarbonate, (C)  $\text{CO}_2$  tension and (D) pH in arterial blood of *Cryptobranchus alleganiensis* (●—●; means  $\pm$  1 s.e.m.,  $N = 8$ ) and *Bufo marinus* (○—○; means only,  $N = 11$  from McDonald *et al.* 1980) before and after 30 min of enforced activity. Shaded area = exercise period. Time B = initial resting sample. Time 0 = immediately post-exercise. Temp. = 25 °C.

similar re-establishment can take up to 10 hours. It is this comparatively large and persistent respiratory acidosis in the salamander which accounts for the dissimilar patterns of acid-base recovery (Fig. 2). Although blood bicarbonate concentrations decrease in both species, the changes in *Cryptobranchus* are of a more complex nature because they represent the sum of two opposing processes which are similar in magnitude; i.e. an increase in  $[\text{HCO}_3^-]$  accompanying the elevation of  $P_{\text{a,CO}_2}$  and a decline in  $[\text{HCO}_3^-]$  resulting from the additions of metabolic acids (principally lactic



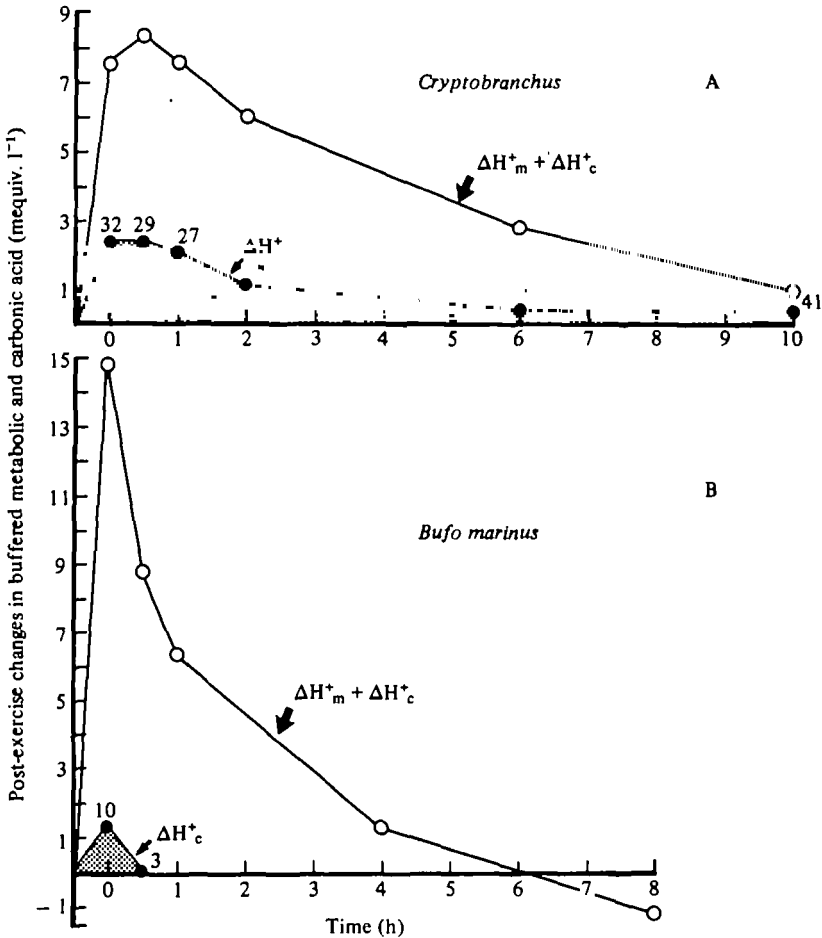


Fig. 3. Calculated post-exercise changes (means  $\pm$  1 S.E.M.) of buffered metabolic acid ( $\Delta H_m^+$ ) and carbonic acid ( $\Delta H_c^+$ ) in arterial blood of 8 *Cryptobranchus alleganiensis* (A) and 11 *Bufo marinus* (B; data from McDonald *et al.* 1980). The upper line of each plot (O) represents the sum total of buffered metabolic and respiratory added acids ( $\Delta H_m^+ + \Delta H_c^+$ ). Lower lines (●) and shaded areas represent the proportion of carbonic acids only ( $\Delta H_c^+$ ). Numbers above each dot enclosing the shaded areas represent the relationship: ( $\Delta H_c^+ / \Delta H_m^+ + \Delta H_c^+$ ), 100%. All values for buffered acids are in m-equiv of H<sup>+</sup> ions/litre of whole blood. Time 0 = immediately post-exercise. Temp. = 25 °C.

acid) to the blood. This point is further illustrated in Fig. 3 where a Davenport analysis, based on measurements of pH, total CO<sub>2</sub> and *in vitro* blood buffering capacity ( $\Delta[HCO_3^-]/\Delta pH$ ), has been used to quantify the respiratory and metabolic acid components of the post-exercise acidosis in *Cryptobranchus* and *Bufo*. The upper line of each plot in Fig. 3 represents the total calculated acid (i.e. the sum total of buffered hydrogen ions added by respiratory and metabolic acids) that appeared in the bloodstream at any one time during the period following activity. The lower shaded areas represent those corresponding portions of the total which can be attributed only to the rise in  $P_a, CO_2$  (i.e. the carbonic acid component). The numbers above each sampling period of the shaded areas represent the percentage to which

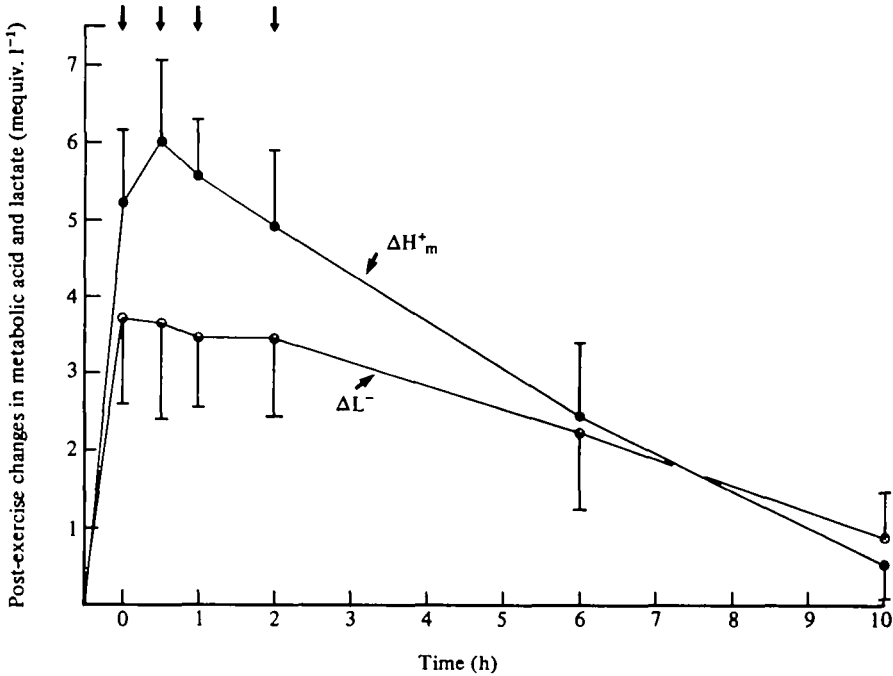


Fig. 4. Post-exercise changes in calculated quantities of metabolic acid ( $\bullet = \Delta H_m^+$ ; in m-equiv of  $H^+$  ions/litre of whole blood) and measured quantities of lactate anion ( $\circ = \Delta L^-$ ; in m-equiv/litre of whole blood) in arterial blood of *Cryptobranchus alleganiensis* at 25 °C (means  $\pm$  1 s.e.m.;  $N = 8$ ). Time 0 = immediately post-exercise. Arrows at top of figure indicate when there is a significant difference ( $P < 0.01$ ) by Student's paired  $t$ -test between  $\Delta$ lactate and  $\Delta H_m^+$ .

the respiratory acid contributes to the total. Clearly, this contribution in *Bufo* is both small and short-lived whereas in *Cryptobranchus* the large and persistent apportionment of carbonic acid appears to underline the interspecific differences of pattern and rate of recovery.

It was earlier mentioned that one criterion for our comparisons of these two species was to induce an arterial blood acidosis of similar magnitude. While it is true that the  $pH_a$  declines were similar (Fig. 2D), the data as assessed in Fig. 3 show that the overall acidosis at time zero in *Cryptobranchus* ( $7.61 \pm 1.13$  m-equiv.l<sup>-1</sup>) was only one-half of that seen for *Bufo* ( $14.7 \pm 1.0$  m-equiv.l<sup>-1</sup>). This smaller acidosis for a similar depression in  $pH_a$  reflects the generally lower blood buffering capacities of *Cryptobranchus* relative to *Bufo*. An acidosis of similar magnitude to that average seen in *Bufo* caused a fatal acidosis in one of the *Cryptobranchus* used in the pilot experiments.

Post-exercise changes in the arterial blood concentrations of metabolic acid (calculated) and lactate (measured) are similar in direction but differ quite markedly in quantitative detail over the first 2 h of recovery in *Cryptobranchus* (Fig. 4). During this time, lactate can account for only 60–70% of the metabolic acid quantity. At the 6th and 10th hour of recovery, these quantities were at parity with one another. A similar discrepancy was found to occur over the first hour of post-exercise in *Bufo* (Table 1 in McDonald *et al.* 1980).

## DISCUSSION

In anuran amphibians such as the bullfrog and toad, evidence has accumulated to suggest that while the skin is responsible for eliminating the major portion of metabolic  $\text{CO}_2$  produced under resting conditions, the control of  $\text{CO}_2$  losses probably resides with pulmonary ventilation (see Introduction for references). *Cryptobranchus*, however, depends almost entirely on its skin for respiratory gas exchange, with more than 97% of the animal's  $\dot{V}_{\text{CO}_2}$  being so liberated at temperatures ranging from 5 to 25 °C (Guimond & Hutchison, 1973). Furthermore, when *Cryptobranchus* is exposed to hypercapnea, it is unable through an increase in lung ventilations to reduce the  $P_{\text{CO}_2}$  difference between arterial blood and ambient medium (Boutilier, 1978) in contrast to the toad (Boutilier *et al.* 1979a, b). Although the number of air breaths increased with activity (Fig. 1A), it must be remembered that the lungs are poorly vascularized sacs, and that the ventilatory frequency was at least an order of magnitude less than that seen in the toad (McDonald *et al.* 1980). In addition, a twofold increase of  $P_{\text{a,CO}_2}$  persisted throughout the period of increased ventilatory activity (0–1 h; Fig. 2C). Taken together, these observations suggest that *Cryptobranchus* is unable to regulate  $P_{\text{a,CO}_2}$  rapidly by lung ventilation but rather must rely on adjustments which promote cutaneous  $\text{CO}_2$  losses. A respiratory control system in the cutaneous exchange site would necessarily incorporate either a method for convecting the ambient medium and/or a process for selectively perfusing the skin circuit. Whilst the rocking movements mentioned earlier might serve as a method for convection, it seems unlikely that such a behaviour could reach the efficiency levels required to correct any major changes in the respiratory acid-base variables. This leaves perfusion as the most likely candidate although here again, from the available data on an exclusively skin breathing plethodontid salamander *Desmognathus fuscus* (Piiper *et al.* 1976), it would appear that because the skin's resistance to diffusive transfer is comparatively high, perfusion adjustments will have relatively little effect on cutaneous gas exchange. Whether this situation is applicable to *Cryptobranchus*, however, awaits further experimental evidence. What does seem clear, nonetheless, is that if a respiratory control mechanism does exist in the skin of *Cryptobranchus*, it does not match the capacities seen in the anuran, at least during the period of elevated tissue  $\text{CO}_2$  output seen in the present studies (Figs. 2 and 3).

Strenuous activity in *Cryptobranchus* and *Bufo* provoked increases in aerobic and anaerobic metabolism resulting in the addition of respiratory and metabolic acids to the bloodstream. As a consequence of the rise in tissue metabolism, we can assume that venous blood  $P_{\text{CO}_2}$  necessarily becomes elevated, a response long since documented for exercising man (Laug, 1934) and one which has more recently been observed in ectothermic vertebrates such as teleost fish (Stevens & Randall, 1967; Wood *et al.* 1977). The toad, through hyperventilation (McDonald *et al.* 1980), is able to offset any major or long-lasting effect that an elevated tissue metabolism (i.e. increase in  $P_{\text{CO}_2}$ ) might have on arterialized blood  $P_{\text{CO}_2}$  and thus  $\text{pH}_a$  (Figs. 2 and 3). Consequently, the bulk of the post-exercise acidosis is metabolic in origin and, in likeness to man (Keul, Doll & Deppler, 1972), recovery is directed almost exclusively toward the metabolic conversion and/or removal of the organic acid component (Fig. 3B). Conceding the differences in rate, the similarities between the exponential recovery pattern seen in the toad and in man are indeed striking.

In marked contrast is the recovery pattern of the salamander, characterized by a comparatively large and enduring respiratory acid component and a lower magnitude, longer duration metabolic acid component (Fig. 3A). The occurrence of the former suggests that  $P_{a,CO_2}$  regulation in *Cryptobranchus* may be relatively imprecise with  $CO_2$  losses via the skin being only poorly controlled in comparison to the pulmonary system of the toad. The conspicuous respiratory acid constituent in *Cryptobranchus* may serve, however, to explain the strategy behind the slow appearance and/or removal of arterial blood lactate (Fig. 2A).

The lactic acid increase and its subsequent plateau in the blood of *Cryptobranchus* implies that lactate either continues to be produced in the tissues (i.e. after the period of activity) or for some reason is only slowly released from the muscles into the bloodstream. Of these two possibilities, the former seems most unlikely and it is not in agreement with experimental results obtained by Hutchison & Turney (1975), Cushman *et al.* (1976) or Preslar & Hutchison (1978), all of whom found that lactate contents of amphibian muscle tissues (*Rana*, *Ambystoma* and *Amphiuma* respectively) decreased from the beginning of recovery following 30 min of enforced activity. Alternatively, the slow time course of post-exercise changes of blood lactate may be attributable to conditions of low capillary density (Foxon, 1964) and/or perfusion rates of the tissues in which lactate has been produced or to which lactate is transferred. If the transfer of lactate from the working muscles to the bloodstream is perfusion limited, it could account for the rather small cardiovascular adjustment seen following exercise in *Cryptobranchus*. Presumably, an increased perfusion of the skeletal musculature at this time would be at the expense of cutaneous blood flow and thus the dominant site for respiratory gas exchange. This, together with a more rapid appearance of lactate, would serve only to increase the magnitude of the overall acidosis. One distinct advantage of a perfusion limitation, however, would be that the total quantity of lactate produced during activity would not coincide in whole with the respiratory acidosis. Considering the magnitude of the respiratory acid component (Fig. 3A) together with the implications of a rapid lactate efflux leads to the conclusion that their synchrony could very likely result in an irreversible acidosis. In contrast, the comparatively large cardiovascular adjustment in *Bufo* (McDonald *et al.* 1980) may underline both the rapid appearance of lactate (i.e. vasodilation of the resistance vessels in working muscle) and the efficiency with which  $P_{a,CO_2}$  is controlled (i.e. ventilation/perfusion relationships). It seems reasonable to conclude, therefore, that the fundamental difference in lactate kinetics between these two species pivots upon the expediency with which the aerobic component of the acid-base disturbance (increase  $P_{a,CO_2}$ ) can be neutralized.

Because the present studies were performed at 25 °C, we can eliminate temperature dependence as one variable which could have resulted in the differential appearances of lactate (Fig. 2A). This is not to say that thermal effects, at both the inter- and intraspecific levels, will not influence the rates of intracellular lactic acid production and removal, but it does point to one example where factors other than temperature (i.e. diffusion and perfusion) must be governing the differential efflux rates at which lactate enters the bloodstream (see McDonald *et al.* 1980). Relating similar considerations to the situation seen in strenuously exercised fish, Wood *et al.* (1977) have pointed out that many of the previously reported inconsistencies between the

time sequence of blood lactate and pH may have been largely due to an essentially mammalian interpretation of the data; i.e. the possible influences of aerobic metabolism on blood  $P_{CO_2}$ , were ignored. Interestingly, the post-exercise recovery pattern in the venous blood of the flounder (Wood *et al.* 1977) and in the arterial blood of the dogfish (Piiper, Meyer & Drees, 1972) is not unlike that observed in *Cryptobranchus*, in that the slow time course of lactic acid release can be interpreted as a strategy which avoids the coalition between peak respiratory and metabolic effects. One common element of these latter studies is the development of a marked respiratory acidosis immediately following the period of activity. It may be, therefore, that such recovery strategies can be correlated with an animal's ability to regulate its arterial blood  $P_{CO_2}$ , and thus  $pH_a$ . While conceding the small number of species studies to date, the available data do suggest this to be one feature which may have influenced the strategies employed for lactate efflux rates at the level of the tissues.

Finally, the apparent large discrepancy between the quantities of metabolic acid and lactate over the first 2–4 h of post-exercise in *Cryptobranchus* is similar to that seen in *Bufo*. Possible reasons for the disparity are discussed by McDonald *et al.* (1980) but it seems worth mentioning that similar discrepancies have been observed in animals ranging from the crab to the human (Osnes & Hermansen, 1972; Wood *et al.* 1977; Benadé & Heisler, 1978; McDonald *et al.* 1979) which suggests that it may be a biological phenomenon inherent in animal tissue.

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