ACID-BASE BALANCE AND TEMPERATURE IN
A PREDOMINANTLY SKIN-BREATHING SALAMANDER,
CRYPTOBRANCHUS ALLEGANIENSIS *

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Abstract. Blood gases and pH and plasma [Na\(^+\)], [K\(^+\)], [Cl\(^-\)] and [lactate] were measured on arterial blood of the large predominantly skin-breathing salamander, the hellbender (Cryptobranchus alleganiensis), at 5, 15 and 25 °C, both with and without access to air. Access to air had no effect on any of the acid–base variables, but temperature had significant effects on both pH and P\(_{\text{CO}_2}\). Blood pH decreased with temperature by about 0.016 unit/°C both in vivo and in vitro (over the range studied) which is similar to the change previously observed on other ectotherms. Blood P\(_{\text{CO}_2}\) rose significantly with temperature while plasma [HCO\(_3^{-}\)] rose slightly but insignificantly. Other ions were unaffected by temperature. This is the first demonstration that the characteristic ectothermic acid-base response to temperature occurs in a vertebrate respiring exclusively through its skin.

We suggest that the response in this animal is essentially passive and uncontrolled and is due to:

1. the proportional effects of temperature upon metabolic CO\(_2\) production and blood P\(_{\text{CO}_2}\), and
2. the temperature-independent CO\(_2\) conductance of the skin.

The skin is an important site for gas exchange in many vertebrates, especially the Amphibia. In some amphibians the lungs are either absent or are present as simple sacs so that all or most of the O\(_2\) and CO\(_2\) exchange is cutaneous. Because of the importance of CO\(_2\) loss in acid–base balance, the skin of these animals clearly has potential importance as the site for control of blood P\(_{\text{CO}_2}\) and pH.

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Recent evidence, however, indicates that cutaneous CO₂ loss in amphibians may be poorly controlled. In the bullfrog, *Rana catesbeiana*, the skin diffusion capacity for CO₂ was unaffected by changes in metabolic rate (Gottlieb and Jackson, 1976), by changes in body temperature (Mackenzie and Jackson, 1978) or by CO₂ breathing (Jackson and Braun, 1979). Boutilier *et al.* (1980) found that the predominantly skin-breathing salamander, the hellbender (*Cryptobranchus alleganiensis*), had a marked elevation in blood $P_{\text{CO}_2}$ following a period of forced activity, and that the $P_{\text{CO}_2}$ returned very slowly to normal. These observations suggest that the regulation of pH associated with respiratory control of $P_{\text{CO}_2}$ may be absent in primarily skin-breathing amphibians.

What of the important pH adjustment that occurs in ectothermic vertebrates when body temperature is altered? (Rahn, 1966). In vertebrates generally, physico-chemical effects on equilibria reactions in the blood cause pH to decrease by about 0.016-0.020 unit per °C increase in body temperature ($dpH/dT = -(0.016 \text{ to } 0.020)$ unit/°C). These effects have been studied *in vitro* (Rosenthal, 1948; Reeves, 1972, 1976), but occur in the animal as well when its body temperature changes. In air-breathing ectotherms, such as turtles, that possess effective pulmonary exchange, this physically-determined shift in pH is preserved and regulated by the respiratory control of $P_{\text{CO}_2}$ (Jackson *et al.*, 1974). In fish, on the other hand, the $dpH/dT$ relationship is regulated primarily by an ionic transport mechanism that alters plasma ($\text{HCO}_3^-$) (Cameron, 1978). This pH-temperature relationship has not previously been investigated in an animal employing only cutaneous gas exchange. It is of interest because the apparent absence of an effective mechanism for the physiological control of pH makes it unclear how the skin breather can match the pH control of other ectotherms.

To investigate this problem, we have determined the arterial acid–base status of the hellbender as a function of temperature. This animal has been shown to rely almost exclusively on its skin for CO₂ elimination (Guimond and Hutchison, 1973).

Materials and Methods

**ANIMALS**

Hellbenders (*Cryptobranchus alleganiensis*) were collected in Missouri in October, 1979 and shipped to us in excellent condition. They were maintained unfed in aerated tap water at 3 °C, with a 12-12 photoperiod, until they were studied in January, 1980. The 10 animals used in experiments ranged in body mass from 272-720 ($\bar{x} \pm SE = 412 \pm 43$) g.
Arterial catheterization was performed on animals that were anesthetized by immersion in a solution of 0.035% tricaine methanesulfonate (Finquel, Ayerst), neutralized with NaOH to a pH of 7.8 at room temperature (Boutilier et al., 1980). An incision (about 2 cm) was made through the pectoral girdle to expose the aortic arches. An electrosurgical knife (Birtcher, Model 755) was used in order to minimize bleeding from the richly vascularized skin. A catheter (PE 50 or PE 90) was placed into either the first or second arch, whichever was larger, and secured with silk sutures. The catheter was led out of the animal through the muscle and skin, and each of these tissue layers was sewn shut separately and secured to the catheter. The catheter was flushed and filled with heparinized (10 u/ml) amphibian Ringer’s solution (Adrian, 1956). The animals were rinsed and placed in fresh water at room temperature until fully recovered. Recovery was apparently complete within 4 hours.

EXPERIMENTAL PROTOCOL

Following recovery from the operation, the animals were placed in an aerated water bath at either 5, 15 or 25 °C with access to air. After at least one day, blood samples were taken and analyzed for ions, blood gases and pH (see below for methods). This sampling procedure was repeated on one or more successive days under the same conditions to ensure that a steady-state acid–base condition existed. A screen was then placed over the water surface to deny the animal access to atmospheric air, although water aeration was continued. After one day without breathing, additional samples were taken at daily intervals. In 5 of the 10 animals studied, this procedure was carried out at 2 different temperatures (3 in the sequence 5–15 °C and 2 in the sequence 25–15 °C). The other 5 animals were studied at only 1 temperature each: 1 at 5 °C, 2 at 15 °C and 2 at 25 °C. In order to avoid possible hypoxia at 25 °C, the water was bubbled with pure O₂ when the hellbenders were submerged.

Experiments had to be terminated between 4 and 10 days after catheterization because of the deteriorating condition of the animals. This was frequently associated with a drastic fall in hematocrit, that could not be accounted for by blood sampling, or by internal bleeding as assessed by postmortem examination. Analysis of variance showed that there was no significant effect (at the 95% level) of hematocrit on any of the measured variables when hematocrits were 10% or above; therefore data from animals with hematocrits below 10% were discarded.

Blood analysis

Blood pH, Pₐ₉, and Pₒ₂, were measured on 0.3 ml samples, drawn anaerobically into heparinized glass syringes and injected into a Radiometer BMS3 Mk2 Blood
Micro System thermostatted at the same temperature as the experimental water bath. Additional samples of about 0.4 ml were taken without heparin into plastic syringes and analyzed for hematocrit, plasma [lactate] (Sigma No. 826-UV), plasma [Na⁺] and [K⁺] (Instrumentation Laboratory model 143 Flame Photometer) and plasma [Cl⁻] (Radiometer CMT10 Chloride Titrator).

Plasma [HCO₃⁻] was calculated from the Henderson-Hasselbalch equation using values for CO₂ solubility and pK' as given by Reeves (1976). These values at 25°C differ somewhat from the constants determined by Boutilier et al. (1980) for Cryptobranchus blood at 25°C, but the [HCO₃⁻] values obtained from the two sets of constants are nearly the same. We also checked total plasma CO₂ on 2 of our samples with a Van Slyke micromanometric apparatus and obtained values for [HCO₃⁻] that agreed closely with the calculated values on the same samples. In order to further assess acid-base balance in the hellbenders, we calculated α-imidazole, the fractional dissociation of imidazole (Reeves, 1972), using the pKₐ values from Reeves (1976) and the equation:

\[
\alpha_{\text{im}} = 10^{b/(1 + 10^b)}
\]

where \( b = \text{pH} - \text{pK}_{\text{im}} \).

IN VITRO MEASUREMENTS

Blood samples from one of the animals at 5°C were tested for pH at 5, 15 and 25°C. Two separate blood gas analyzers, each regulated at a different temperature, were employed so that blood sampling and storage artefacts would be minimized.

STATISTICAL ANALYSIS

Differences in the measured in vivo variables due to the two treatment regimes, temperature change and air access status, were evaluated with an analysis of variance computer program, SPSS (Kim and Kohout, 1975).

RESULTS

Both in vivo blood [H⁺] and Pco₂ increased significantly with temperature in the hellbender (table 1) but, with the exception of P O₂, none of the measured variables was influenced by whether access to air was possible or not (tables 1 and 2). The P O₂ effect was due to the experimental elevation of ambient P O₂ at 25°C when the hellbenders were apneic. Because there was no significant difference in blood pH between the air-access vs. no air-access treatments, pHₐ values for both were combined and the mean values at each temperature are plotted as the
Fig. 1. Blood pH of hellbenders at various temperatures with access to air (open circles) and without access to air (closed circles). The solid line connects the pooled mean value at each temperature. The dotted line connects in vitro determinations of a sample collected at 5°C.

solid line in fig. 1. The $\Delta$H/AT values for 5–15°C and 15–25°C are $-0.017$ and $-0.015$ respectively. The corresponding slope for the in vitro line from 5–25°C has an overall value of $-0.016$ and is plotted as the dashed line. This line, although parallel to the in vivo line, is displaced downward because the 5°C in vivo pH of this animal at the time of sampling was at the low end of the range. The conformity of the hellbender's in vivo acid–base balance to typical ectotherm control is confirmed by the constancy of the calculated variable, $\alpha$-imidazole (table 1).

Neither plasma $[Na^+]$ nor $[Cl^-]$ changed significantly with temperature and, although plasma $[K^+]$ did increase significantly, the absolute magnitude was small (table 2). Plasma [lactate] was generally low and variable, but with no consistent relationship to either temperature or air access status. Plasma $[HCO_3^-]$, calculated from $pH_a$ and $Pa_{CO_2}$, was also not significantly influenced by temperature, but the data do reveal a trend for $[HCO_3^-]$ to increase with temperature (table 1).

Discussion

This study demonstrates that temperature affects the in vivo blood pH of Cryptobranchus in the same manner as in most other ectotherms; i.e., pH falls by about 0.016 unit/°C rise in temperature. This is the first confirmation that this response occurs in an animal respiring through its skin without active ventilation of specialized exchange structures such as the lungs or gills. It is of interest therefore to compare how the pH change may be achieved in this animal without the mechanisms utilized by animals with pulmonary or branchial exchange. To do this it is convenient to separate the effects of temperature on the acid–base
# TABLE 1

Blood acid-base status and PO$_2$ of *Cryptobranchus alleganiensis* at various temperatures with and without access to air-breathing

<table>
<thead>
<tr>
<th>Temp. (°C)</th>
<th>Condition</th>
<th>(N,n)$^1$</th>
<th>[H$^+$] (pH) (nM/L)</th>
<th>[HCO$_3^-$] (mEq/L)</th>
<th>P$_{CO_2}$ (Torr)</th>
<th>P$_O_2$ (Torr)</th>
<th>α-Imidazole</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>Access to air</td>
<td>(5,10)</td>
<td>8.10 (8.09)</td>
<td>9.5</td>
<td>2.03</td>
<td>16.9</td>
<td>0.85</td>
</tr>
<tr>
<td></td>
<td>Apneic</td>
<td>(4,5)</td>
<td>9.53 (8.02)</td>
<td>± 0.41</td>
<td>± 0.24</td>
<td>± 0.08</td>
<td>± 1.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>± 1.61</td>
<td>± 1.61</td>
<td>± 0.6</td>
<td>± 0.10</td>
<td>± 2.0</td>
</tr>
<tr>
<td>15</td>
<td>Access to air</td>
<td>(5,13)</td>
<td>12.4 (7.90)</td>
<td>10.7</td>
<td>4.20</td>
<td>17.2</td>
<td>0.85</td>
</tr>
<tr>
<td></td>
<td>Apneic</td>
<td>(4,9)</td>
<td>12.6 (7.90)</td>
<td>± 0.39</td>
<td>± 0.21</td>
<td>± 0.13</td>
<td>± 0.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>± 0.35</td>
<td>± 0.33</td>
<td>± 0.32</td>
<td>± 0.32</td>
<td>± 0.9</td>
</tr>
<tr>
<td>25</td>
<td>Access to air</td>
<td>(4,8)</td>
<td>17.8 (7.75)</td>
<td>11.7</td>
<td>± 1.9</td>
<td>± 0.58</td>
<td>± 0.13</td>
</tr>
<tr>
<td></td>
<td>Apneic</td>
<td>(3,3)</td>
<td>18.8 (7.73)</td>
<td>12.7$^2$</td>
<td>8.75$^2$</td>
<td>15.3</td>
<td>0.85</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>± 1.1</td>
<td>± 1.1</td>
<td>± 1.1</td>
<td>± 1.1</td>
<td>± 0.01</td>
</tr>
</tbody>
</table>

Analysis of variance

- Condition: $P < 0.001$
- Temperature: $P < 0.001$
- Temperature × condition: $P < 0.05$

Values are mean ± SE.

$^1$ N is number of animals; n is number of measurements.

$^2$ based on only 2 measurements.
### TABLE 2

Plasma ion concentrations of *Cryptobranchus alleganiensis* at various temperatures with and without access to air

<table>
<thead>
<tr>
<th>Temp. (°C)</th>
<th>Access to air</th>
<th>Apneic</th>
<th>Analysis Condition</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(N,n)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>(4,8)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Access to air</td>
<td>5.4 ± 1.6</td>
<td>1.76 ± 0.07</td>
<td>83.4 ± 0.8</td>
</tr>
<tr>
<td>Apneic</td>
<td>95.4 ± 0.9</td>
<td>2.0 ± 0.2</td>
<td>83.1 ± 2.3</td>
</tr>
<tr>
<td>Access to air</td>
<td>5.4 ± 2.3</td>
<td>2.2 ± 0.1</td>
<td>80.1 ± 1.9</td>
</tr>
<tr>
<td>Apneic</td>
<td>95.9 ± 7.4</td>
<td>2.4 ± 0.3</td>
<td>78.2 ± 4.2</td>
</tr>
<tr>
<td>15</td>
<td>(3,3)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Access to air</td>
<td>88.8 ± 1.3</td>
<td>2.6 ± 0.1</td>
<td>71.2 ± 1.7</td>
</tr>
<tr>
<td>Apneic</td>
<td>87.7 ± 2.6</td>
<td>2.5 ± 0.1</td>
<td>72.1 ± 2.1</td>
</tr>
</tbody>
</table>

Values are mean ± SE.

1 N is number of animals; n is number of measurements.

The physical effects of temperature are due to the temperature-dependence of both the weak acid equilibria and the CO$_2$ solubility. These effects have been thoroughly studied on blood *in vitro* as a closed system (Reeves, 1972, 1976). The physical effects are stereotyped and appear to be nearly identical in all vertebrate blood. Briefly, the physical effects of temperature on blood cause pH to decrease with temperature (\( \frac{d\text{pH}}{dT} = -0.016 \text{ to } -0.020 \text{ unit/°C} \)), and P$_{CO_2}$ to increase with temperature (\( \frac{d\log P_{CO_2}}{dT} \approx 0.020 \)) (Reeves, 1977). Because the system is closed, total CO$_2$ content and strong ion concentrations are unchanged. These physical effects must also occur *in vivo* and cause the same effects on the blood of animals undergoing body temperature changes. Our measurement of the *in vitro* effect of temperature on the pH of the hellbender’s blood confirns that this effect occurs in this animal (fig. 1).

The physiological effects influencing acid–base balance result from the metabolic activity of the animal and from the exchange of substances (ions and CO$_2$) between the animal and its environment and between fluid compartments within the animal.
Temperature has less predictable effects on these processes than it does on the physical events, although metabolic rate generally increases with body temperature in ectotherms with a $Q_10$ of about 2–3. Ionic and respiratory exchange processes, however, are affected by temperature differently in different animals depending on the characteristics of the control systems governing these exchanges.

In the hellbender, the *in vivo* blood acid–base response to temperature closely resembled the changes expected from the physical effects alone (table 2). This means that the physiological processes of metabolism and exchange responded to temperature in such a way as to preserve the physically-determined state. This same identity between *in vitro* and *in vivo* blood acid–base response to temperature has been observed in various air-breathing ectotherms, such as the turtle, *Pseudemys scripta* (Robin, 1962) and the bullfrog, *Rana catesbeiana* (Reeves, 1972). In these animals, however, the physiological control of $P_{CO_2}$ via pulmonary ventilation adjusts with temperature to regulate acid–base balance (Jackson et al., 1974; Mackenzie and Jackson, 1976). The hellbender, in contrast, relies upon its skin for respiratory $CO_2$ exchange and there is recent evidence that indicates that skin gas exchange is poorly controlled.

In our laboratory, we have studied the control of skin $CO_2$ loss in the bullfrog, *Rana catesbeiana*, by determining the total skin conductance for $CO_2$ under various circumstances, including changes in body temperature. Total conductance ($G_{CO_2}$) was calculated as the rate of $CO_2$ loss across the skin divided by the difference between arterial and ambient $P_{CO_2}$ ($dP_{CO_2}$). We found that $CO_2$ loss was the same function of $dP_{CO_2}$ at 10, 20 and 30°C; *i.e.*, $G_{CO_2}$ remained unchanged over this temperature range in the bullfrog. In their recent analysis of skin gas exchange in amphibians, Piiper and Scheid (1977) subdivided the total conductance into 3 components: perfusive conductance, diffusive conductance and ventilatory conductance. Because the amphibian skin is exposed to ambient $P_{CO_2}$, the ventilatory conductance is considered in their model to be infinite and invariant, and therefore not a controllable factor. Furthermore, an analysis by this same laboratory of cutaneous gas exchange in the lungless salamander, *Desmognathus fuscus* (Piiper et al., 1976) led to the conclusion that perfusive conductance exceeds diffusive conductance by some 5-fold. In other words, skin gas exchange is a diffusion-limited system in which variations in blood perfusion have little effect. Because diffusion *per se* is only weakly temperature-dependent with a $Q_10$ of about 1.1 (Dejours, 1975), this analysis is consistent with physiological observations on the bullfrog cited above. Finally, in the recent study by Boutilier *et al.* (1980), the acid–base consequences of induced activity were compared in the hellbender and in the toad, *Bufo marinus*. Their results revealed that the hellbender exhibited not only a metabolic (lactic) acidosis, but also a severe respiratory acidosis which subsided slowly after the termination of activity. In contrast, the toad, with its more effective pulmonary ventilation, had elevated lactic acid but a milder respiratory acidosis that was promptly restored to normal during recovery. This pronounced $CO_2$ retention by the hellbender during and after activity is consistent
with a poorly controlled CO₂ exchange, and one in which the rate of exchange can be increased primarily by increasing dP_{CO₂}.

We suggest, therefore, that what appears to be acid-base control in the hellbender in response to temperature change is in reality simply a close matching between the physical effects of temperature and the largely uncontrolled physiological effects of temperature. Consider the consequences of a 10°C increase in body temperature from 5 to 15°C. At 5°C, under steady-state conditions, the CO₂ loss of the hellbender (equal to its metabolic CO₂ production) occurs by diffusion across its skin with a dP_{CO₂} of about 2 Torr (table 1). When its body temperature rises to 15°C, the physical effects on the blood cause pH to fall by about 0.17 unit and P_{CO₂} to rise to about 4 Torr. The increase in temperature also stimulates an increase in metabolic CO₂ production of about 2-fold (Guimond and Hutchison, 1976). There is thus twice as much CO₂ to be lost by diffusion across the skin, but the dP_{CO₂} is also approximately double and, assuming no change in total skin conductance, the system is automatically in a new steady-state with respect to CO₂ exchange. These events occur with no significant CO₂ retention. Apparently, this essentially passive, uncontrolled acid-base response can occur over the temperature range between 5 and 25°C (fig. 2).

The possibility of some control of gas exchange in the hellbender cannot be excluded, however, even during exclusively cutaneous respiration. Many observers have noted the rocking or swaying motion that these animals employ in warm or still water (Nickerson and Mays, 1972). We also observed this in our animals, particularly in 25°C water. Presumably, this movement should serve to decrease the unstirred boundary layer at the skin surface and thereby facilitate gas exchange (Guimond and Hutchison, 1973), although no experimental verification of this is

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**Fig. 2.** Mean values of arterial P_{CO₂} from the present study and of skin CO₂ loss (V_{CO₂}) from Guimond and Hutchison (1976) measured in hellbenders at various temperatures. Skin CO₂ conductance (G_{CO₂}), the ratio between V_{CO₂} and P_{CO₂}, has been calculated from these two sets of data, and appears to be independent of body temperature.
available. In view of our circumstantial evidence for unchanged total skin conductance, and the data of Boutilier *et al.* (1980) on the prolonged elevation of PaCO₂ following activity, it would appear that this control is relatively ineffective at least for CO₂ elimination, although its effects on O₂ transfer remain unknown. It should be noted that the hellbenders studied by Boutilier *et al.* (1980) had access to air, and were observed to increase their respiratory rate following activity, and still a sustained hypercapnia was observed.

*In conclusion,* our results, together with previous work on the hellbender and on other amphibians, indicate that skin CO₂ loss is poorly controlled and can be increased significantly only by increasing blood PₐCO₂. Consequently, blood PₐCO₂ in an animal such as Cryptobranchus, that relies primarily on its skin for CO₂ loss, must fluctuate with metabolic rate. At constant temperature this fluctuation produces a respiratory acid–base disturbance. However, when metabolic rate varies strictly as a result of body temperature change, the associated fluctuations in blood PₐCO₂ do not reflect changes in CO₂ content and are appropriate for the maintenance of normal ectothermic acid–base balance.

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**References**


Kim, J.-O. and F. J. Kohout (1975). Analysis of variance and covariance: subprograms anova and


