

## COMPARISON OF DIRECTLY DETERMINED AND CALCULATED PLASMA BICARBONATE CONCENTRATION IN THE TURTLE *CHRYSEMYS* *PICTA BELLII* AT DIFFERENT TEMPERATURES

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In any study on the effects of changes in body temperature on acid-base status of ectotherms, the relationship between temperature and plasma bicarbonate is of central interest. Many such studies report that arterial pH varies inversely with temperature (see reviews by Howell & Rahn, 1976; Reeves & Rahn, 1979; Jackson, 1982); in air-breathing vertebrates, this is often exclusively caused by a rise in blood  $P_{CO_2}$  with body temperature. In contrast, total  $CO_2$  ( $C_{CO_2}$ ) and thus bicarbonate concentration of the plasma remain essentially constant with changes in temperature. Other studies report a departure from this pattern with bicarbonate concentrations changing significantly with temperature (Bennett, 1973; Wood, Glass & Johansen, 1977; Nolan & Frankel, 1982). However, in most studies on acid-base balance in reptiles, bicarbonate concentration has not been measured directly but has been calculated from the Henderson-Hasselbalch equation:

$$[HCO_3^-] = 10^{(pH - pK'_1)} \cdot P_{CO_2} \cdot \alpha_{CO_2}, \quad (1)$$

where  $pK'_1$  is the apparent first dissociation constant of the bicarbonate buffer system and  $\alpha_{CO_2}$  is the solubility of  $CO_2$  in plasma.

Such calculations require selection of suitable values for the parameters  $pK'_1$  and  $\alpha_{CO_2}$ , both of which vary with temperature. These values have often been calculated from the polynomial functions presented by Reeves (1976), derived from data given by Severinghaus (1965) for mammalian blood:

$$pK'_1 = 6.3852 - 1.3288 \cdot 10^{-2}t + 1.7364 \cdot 10^{-4}t^2 - 6.0084 \cdot 10^{-7}t^3 \quad (2)$$

$$\alpha_{CO_2} = 0.0907 - 0.3373 \cdot 10^{-2}t + 0.6749 \cdot 10^{-4}t^2 - 5.4076 \cdot 10^{-7}t^3, \quad (3)$$

(mmol mmHg<sup>-1</sup>)

where:  $t$  = temperature (°C).

These functions, however, do not provide any correction for the actual pH in spite of the fact that  $pK'_1$  is affected by pH as well as by temperature (Siggaard-Andersen, 1976; Severinghaus, 1965). The effect of pH on  $pK'_1$  is taken into account by the polynomial function presented by Siggaard-Andersen (1974), which was derived on

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the basis of data of Harned & Davis (1943), Maas, van Heijst & Visser (1971) and Siggaard-Andersen (1962):

$$pK'_1 = 6.125 - \log(1 + 10^{pH-8.7}) - 0.0026(t - 37) + 0.00012(t - 37)^2. \quad (4)$$

The bicarbonate concentration calculated based on measured pH and  $P_{CO_2}$  values will be greatly affected by errors in  $pK'_1$ . This was clearly demonstrated by Howell & Rahn (1976) who showed that the plasma bicarbonate concentration of turtles calculated using Reeves'  $pK'_1$  values, was relatively constant as temperature increased, whereas bicarbonate calculated using  $pK'_1$  values from Severinghaus (1965) decreased by 14%/10°C.

However, the absolute accuracy of the  $pK'_1$  values calculated from the equations of Reeves and Siggaard-Andersen have never been checked and neither have the changes of  $pK'_1$  with temperature and actual pH. Therefore, the aim of the present study was to determine directly the three variables of the Henderson-Hasselbalch equation (pH,  $P_{CO_2}$  and  $[HCO_3^-]$ ) in the plasma of turtles acclimated to a range of temperatures and to compare the obtained  $pK'_1$  values and bicarbonate concentrations with those derived from the above equations.

Semi-aquatic turtles of the species *Chrysemys picta bellii* were kept in captivity for several months prior to the experiments. Animals were housed in large glass aquaria equipped with heat lamps and basking areas. Eleven turtles, average weight 490 g (range 391–617 g) were used in these experiments.

Turtles were cannulated by a procedure similar to that described by Jackson, Palmer & Meadow (1974). A 3.2-cm diameter hole was cut in the plastron, exposing the left brachial artery. A PE50 Polythene catheter with side holes at the tip was inserted into the artery and tied in, fed out behind the leg, and then fixed to the carapace with tissue glue and tape. The excised plastron plug was replaced and secured with dental acrylic. Catheters were flushed with saline and filled with heparinized saline (100 i.u. ml<sup>-1</sup>). Approximately 6 h after recovery from anaesthesia, the turtle was placed in a thermostatically controlled aquarium at 10, 20 or 30°C. The aquarium contained water to a depth of 2–4 cm so that the turtle was not forced to swim, and was placed in a large insulated box to minimize disturbance. Air was pre-heated or cooled to the aquarium temperature and bubbled through the water.

Animals were given at least 18 h to equilibrate to the experimental temperature and then blood samples were taken anaerobically into heparinized syringes and immediately analysed for  $P_{CO_2}$ ,  $P_{O_2}$  and pH by means of electrodes thermostatted to the respective body temperature (BMS 3, Radiometer, Copenhagen). The  $P_{CO_2}$  and  $P_{O_2}$  electrodes were calibrated before and after each measurement with humidified gas mixtures from gas mixing pumps (Wösthoff, Bochum FRG). The pH electrode was calibrated with precision phosphate buffers (S1500 and S1510, Radiometer, Copenhagen). Aliquots of each blood sample were centrifuged in microhaematocrit tubes (80 µl), and, after measurement of the haematocrit, total  $CO_2$  content of the plasma was determined using a 'Capni-Con II' total  $CO_2$  analyser (Cameron Inst. Co., Port Aransas, Texas, U.S.A.). In this apparatus all  $CO_2$  species in the plasma sample were converted by dilute acid to dissolved  $CO_2$ , which is removed from the solution by a nitrogen carrier gas stream and then reabsorbed in a dilute NaOH solution. The amount of carbonate thus formed is measured by the change in electrical

conductivity of the NaOH solution. The principles of this method are described in detail by Maffly (1968).

As a check on the accuracy of this method 10 analyses were made on sodium bicarbonate solutions of 35 mM and 50 mM, this being the range of values found in this study. Results were  $35.12 \pm 0.26$  mM and  $49.96 \pm 0.31$  mM, respectively ( $\bar{x} \pm$  s.d.).

Bicarbonate concentration of the plasma was calculated from the relationship  $[\text{HCO}_3^-] = C_{\text{CO}_2} - \alpha_{\text{CO}_2} \cdot P_{\text{CO}_2}$  using  $\alpha_{\text{CO}_2}$  values from the literature (Reeves, 1976).

The  $\text{pK}'_1$  value for each sample was calculated from the Henderson-Hasselbalch equation:

$$\text{pK}'_1 = \text{pH} - \log \left[ \frac{C_{\text{CO}_2}}{\alpha_{\text{CO}_2} \cdot P_{\text{CO}_2}} - 1 \right]. \quad (5)$$

Animals with low haematocrits (less than 16 %) were excluded from the experiments. The ionic strength determined in plasma of turtles of the same batch as the experimental animals was determined from the sum of all plasma electrolytes ( $\mu \sim 0.15$ ).

The results of the blood gas determinations are summarized in Table 1. These data are similar to values recorded for *Chrysemys picta bellii* by Glass, Boutilier & Heisler (1984), the only significant difference being a slightly lower pH ( $7.726 \pm 0.009$ ) at 20°C in the earlier study.

Table 2 compares the  $\text{pK}'_1$  values determined from the acid-base data at each

Table 1. *The effects of temperature on the acid-base status of arterial blood plasma of Chrysemys picta bellii*

Temperature (°C)	pH	$P_{\text{CO}_2}$ (Torr)	$C_{\text{CO}_2}$ (mM)	$P_{\text{O}_2}$ (Torr)	Ht (%)
10 ( $N=23$ )	$7.847 \pm 0.016$	$16.0 \pm 0.72$	$43.2 \pm 0.82$	$20.4 \pm 4.4$	$20.8 \pm 0.43$
20 ( $N=17$ )	$7.778 \pm 0.009$	$22.9 \pm 0.68$	$44.3 \pm 0.77$	$68.6 \pm 4.2$	$17.4 \pm 0.42$
30 ( $N=20$ )	$7.628 \pm 0.006$	$35.2 \pm 0.86$	$43.3 \pm 0.53$	$70.8 \pm 2.1$	$21.5 \pm 0.54$

$\bar{x} \pm$  s.e.;  $N$  = number of observations; Ht = haematocrit.

Table 2. *Comparison of  $\text{pK}'_1$  values determined from the data summarized in Table 1, and the corresponding values from Reeves (1976) and Siggaard-Andersen (1976)*

Temperature (°C)	$\text{pK}'_1$			$[\text{HCO}_3^-]$ (mM)		
	Determined (this study)	Reeves (1976)	Siggaard-Andersen (1974)	Measured (this study)	Based on Reeves (1976)	Based on Siggaard-Andersen (1974)
10	$6.219 \pm 0.055$	6.269	6.226	$42.2 \pm 0.69$	$37.6 \pm 0.69^{***}$	$41.5 \pm 0.74$ NS
20	$6.163 \pm 0.003$	6.184	6.155	$43.2 \pm 0.73$	$41.1 \pm 0.75^{***}$	$44.0 \pm 0.80^*$
30	$6.096 \pm 0.005$	6.127	6.114	$42.1 \pm 0.51$	$39.2 \pm 0.61^{**}$	$40.4 \pm 0.62^{**}$

Also shown is a comparison between measured plasma bicarbonate and values calculated from the Reeves and Siggaard-Andersen  $\text{pK}'_1$  values ( $\bar{x} \pm$  s.e.).

Corresponding measured and calculated values have been compared using a paired  $t$ -test.

N.S. = not significantly different from measured value.

\* Difference from measured value significant at 5 % level.

\*\* Significant at 1 % level.

\*\*\* Significant at 0.1 % level.

temperature with the corresponding values calculated from the polynomial functions given by Reeves (1976) and Siggaard-Andersen (1974). The significance of the differences between the experimentally determined and the calculated  $pK'_1$  values is shown by the comparison of the experimentally determined bicarbonate values\* with values calculated from the same raw data using the Reeves and Siggaard-Andersen  $pK'_1$  values. Corresponding individual concentrations were compared using the paired sample *t*-test. Bicarbonate concentrations calculated from Reeves  $pK'_1$  values were significantly lower than the measured bicarbonate values at all temperatures. The  $pK'_1$  values derived from the Siggaard-Andersen equation produced a closer fit to the experimental data, particularly at the lower temperatures. For this reason the equation presented by Siggaard-Andersen (1974) seems to be the better alternative when it is necessary to calculate  $[HCO_3^-]$ , in spite of the fact that this equation was originally designed for application relatively close to the normal mammalian temperature of 37°C. (It should be noted that at pH = 7.4 the above equation will produce the same  $pK'_1$  values as the polynomial function presented by Reeves.)

Interestingly, the measured bicarbonate values show that temperature has even less effect on plasma  $[HCO_3^-]$  than could be assumed from calculated values. But the absence of any correlation between temperature and  $[HCO_3^-]$  does not mean that  $[HCO_3^-]$  is always constant. Bicarbonate levels seem to vary greatly between individual turtles of the same species so that in the present study at 10°C one turtle maintained  $[HCO_3^-]$  between 46 and 49 mM over a 5-day period of measurement while another had concentrations between 36 and 39 mM over a similar period. These large differences between individuals would obscure any small changes in  $[HCO_3^-]$  as a function of temperature when data from different turtles are grouped together.

The overall  $\Delta pH/\Delta t$  value *in vivo* for the range of 10–30°C was  $-0.0109$  U/°C; from 20 to 30°C,  $\Delta pH/\Delta t$  was higher and near the value for constant relative alkalinity of  $-0.016$  U/°C, whereas over the range 10–20°C,  $\Delta pH/\Delta t$  was only  $-0.0069$  U/°C. *In vitro*,  $\Delta pH/\Delta t$  was checked during the same experiments by taking blood from turtles at 10 and 20°C and measuring the pH of the samples by means of two pH electrodes, thermostatted to 10°C and 20°C, respectively. This procedure resulted in a value for  $\Delta pH/\Delta t$  of  $-0.0184 \pm 0.0028$  ( $\pm$  s.e.,  $N = 4$ ), similar to that reported before for both ectotherms and endotherms (cf. Reeves & Rahn, 1979).

Constancy of plasma  $[HCO_3^-]$  is central to the alaphastat hypothesis of acid-base regulation in air-breathing ectotherms (Jackson, 1982; Reeves & Rahn, 1979), and hence these results would at first seem to support it, particularly when viewed with the results of Hitzig (1982), who showed that in the turtle *Pseudemys scripta elegans* temperature does not affect total  $CO_2$  stores or plasma strong ion difference. Yet the fact that  $[HCO_3^-]$  did not change *in vivo* with temperature, while  $\Delta pH/\Delta t$  was significantly different *in vivo* and *in vitro* indicates that protons were buffered by proteins and that some ionic exchange was taking place between the plasma and other body compartments. Moreover, the overall  $\Delta pH/\Delta t$  value *in vivo* was significantly different from  $\Delta pK_{im}/\Delta t$  at a high level. These facts do not fit well with the alaphastat hypothesis.

\* Values referred to as experimentally determined are always derived from the measured total  $CO_2$  content minus the amount of dissolved  $CO_2$ . Since less than 3% of the total  $CO_2$  content has to be attributed to dissolved  $CO_2$  (cf. Table 2), any error introduced by not quite appropriate  $\alpha_{CO_2}$  values is negligible.

Clearly, a better understanding of acid-base regulation in ectotherms requires more studies in which the acid-base status of other body compartments as well as the blood is examined. However, when the effect of temperature on the acid-base status of the blood is to be investigated, all three parameters ( $\text{pH}$ ,  $\text{P}_{\text{CO}_2}$ ,  $[\text{HCO}_3^-]$ ) of the bicarbonate buffer system should be determined directly. If  $[\text{HCO}_3^-]$  has to be calculated, then, because of the considerable deviation of ectotherm plasma  $\text{pH}$  from 7.4, the  $\text{pK}'_1$  values obtained using the equation presented by Siggaard-Andersen (1974) will result in a better estimate than the commonly used  $\text{pK}'_1$  values based on Reeves (1976).

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