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SHORT COMMUNICATION

DETERMINATION OF THE CONSTANTS OF THE HENDERSON–HASSELBALCH EQUATION, αCO_2 AND pKa, IN SEA TURTLE PLASMA

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Hydration of CO_2 yields HCO_3^- *via* the reaction:

$$CO_2 + H_2O \rightleftharpoons H_2CO_3 \rightleftharpoons HCO_3^- + H^+ \rightleftharpoons CO_3^{2-} + 2H^+. \quad (1)$$

Acid–base physiologists traditionally simplify the reaction by omitting the H_2CO_3 term and lumping all ionic CO_2 species into the HCO_3^- term. The simplified reaction forms the basis for the familiar Henderson–Hasselbalch equation of the CO_2–HCO_3^- buffer system:

$$\mathrm{pH} = \mathrm{pKa} + \log([HCO_3^-]/\alpha CO_2 P_{CO_2}), \quad (2)$$

where αCO_2 is the solubility coefficient relating $[CO_2]$ and P_{CO_2} (Henry's Law). The apparent pK (pKa) in this equation lacks a rigorous thermodynamic definition. Instead, it is an empirical factor relating pH, the product of αCO_2 and P_{CO_2}, and the apparent $[HCO_3^-]$ (i.e. the sum of all ionic CO_2 species).

αCO_2 and pKa are sensitive to the temperature, pH and/or the ionic strength of the reaction medium. αCO_2 and pKa of normal mammalian blood plasma have been well defined over a range of temperatures and pH values (e.g. Severinghaus, 1965; Siggaard-Andersen, 1974; Reeves, 1976). These mammalian values are commonly used in analyses of the acid–base status of non-mammalian species, despite evidence that such practices can produce misleading results (Nicol *et al.* 1983). As an alternative, Heisler (1984; *erratum* in Heisler, 1986) developed complex equations for αCO_2 ($\mathrm{mmol\,l^{-1}\,mmHg^{-1}}$) (1mmHg=133.22Pa) and pKa that are purported to be generally applicable to aqueous solutions (including body fluids) between 0 and 40˚C and incorporate the molarity of dissolved species (M_d), solution pH, temperature (T, ˚C), sodium concentration ($[Na^+]$, $\mathrm{mol\,l^{-1}}$), ionic strength of nonprotein ions (I, $\mathrm{mol\,l^{-1}}$) and protein concentration ([Pr], $\mathrm{g\,l^{-1}}$):

$$\alpha CO_2 = 0.1008 - 2.980\times10^{-2}M_d + (1.218\times10^{-3}M_d - 3.639\times10^{-3})T - (1.957\times10^{-5}M_d - 6.959\times10^{-5})T^2 + (7.171\times10^{-8}M_d - 5.596\times10^{-7})T^3. \quad (3)$$

Key words: turtle, *Lepidochelys kempi*, plasma, αCO_2, pKa.

$$\mathrm{pKa} = 6.583 - 1.341\times10^{-2}T + 2.282\times10^{-4}T^2 - 1.516\times10^{-6}T^3 - 0.341I^{0.323} - \log\{1 + 3.9\times10^{-4}[\mathrm{Pr}] + 10^{A}(1+10^{B})\}\,, \quad (4)$$

where

$$A = \mathrm{pH} - 10.64 + 0.011T + 0.737I^{0.323}$$

and

$$B = 1.92 - 0.01T - 0.737I^{0.323} + \log[\mathrm{Na}^+] + (0.651 - 0.494I)(1+0.0065[\mathrm{Pr}]).$$

Experimental validation of these equations has not appeared in the literature to date.

We determined the αCO_2 and pKa of blood plasma from Kemp’s ridley sea turtles (*Lepidochelys kempi* Garman) and compared the values with those predicted from Heisler’s equations. Blood samples were collected into heparinized syringes from the dorsal cervical sinus of 1- to 2-year-old animals at the National Marine Fisheries Service, Galveston Laboratory, Texas. Separated plasma was obtained by centrifugation of the whole blood samples. αCO_2 was determined gasometrically by equilibrating 2ml samples of acidified plasma (titrated to pH2.5 with 1mol l^{-1} HCl) in a tonometer with 99.9% CO_2 at 20, 25, 30 or 35˚C. Fresh samples of plasma were used at each temperature. The total CO_2 content (C_{CO_2}) of plasma was measured in duplicate after 15min of equilibration, using the methods described by Cameron (1971). The CO_2 electrode (Radiometer, type E5036) was calibrated at each temperature using known [HCO_3^-]. Plasma P_{CO_2} was calculated from the known fractional CO_2 content of the equilibration gas, corrected for temperature, barometric pressure and water vapor pressure. Plasma water content was measured by weighing samples of plasma before and after they had been dried at 60˚C to constant weight. αCO_2 was calculated as the quotient of C_{CO_2} and P_{CO_2}, taking into account the plasma water content (mean ± S.E.= 96±0.03%). pKa was determined gasometrically by equilibrating 2ml samples of plasma in a tonometer with 4.78 or 10.2% CO_2 (balance N_2) at 20 or 30˚C. Fresh samples of plasma were used at each temperature and gas concentration. Plasma C_{CO_2} and pH were measured in duplicate. The pH electrode (Radiometer, type G297/G2) was calibrated at each temperature using precision Radiometer pH buffers (S1500 and S1510). Plasma P_{CO_2} was determined as above. pKa was calculated from a rearrangement of the Henderson–Hasselbalch equation (equation 2), assuming C_{CO_2} to be the sum of [HCO_3^-] and [CO_2] (i.e. $\alpha CO_2 P_{CO_2}$).

Heisler’s equations were adapted for use with *L. kempi* plasma using measured values of the molarity of dissolved species (M_d), [Na^+] and protein concentration ([Pr]). These parameters were quantified as follows: M_d with a vapor pressure osmometer (Precision Systems, model 5004), [Na^+] by flame photometry (Jenway, model PFP7) and [Pr] by a standard spectrophotometric method (Sigma kit 541). The average values were M_d=0.304±0.003mol l^{-1}, [Na^+]=0.141±0.004mol l^{-1} and [Pr]=28±3 g l^{-1}. The ionic strength of nonprotein ions (I) was assigned a value of 0.150mol l^{-1}. Computed αCO_2 and pKa values were generated for a wider range of temperature and pH conditions than were used experimentally in order to emphasize the pattern and range of effects of temperature and/or pH.

Fig. 1A gives the measured values of αCO_2 (mmol l^{-1} mmHg^{-1}) together with the

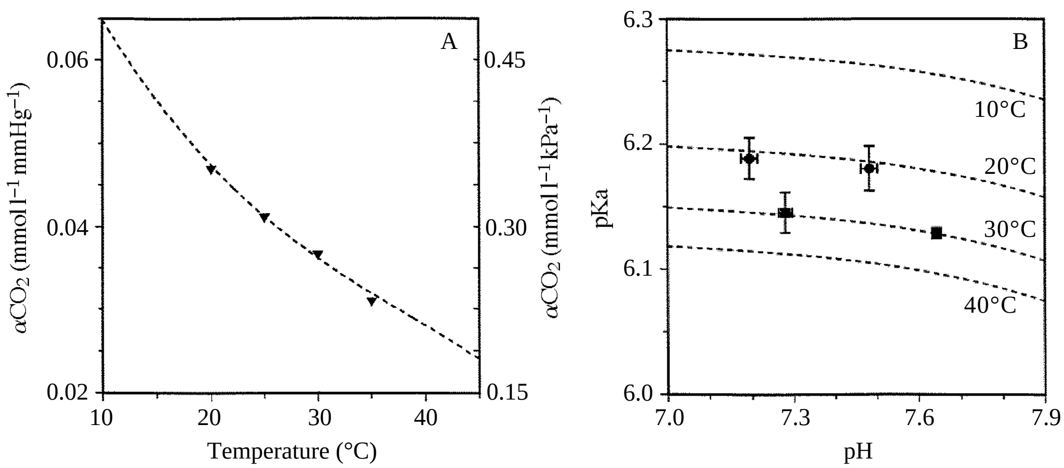

Fig. 1. Experimentally determined values of αCO_2 (A) and pKa (B) for the plasma of *Lepidochelys kempi*. Data points are means ± standard deviation (N=3–9). The absence of error bars indicates that the standard deviation was smaller than the data symbol. Dashed lines give the predicted values of αCO_2 and pKa computed from equations 5 and 6 (see text).

predicted values computed from the following adaptation of Heisler's generalized equation:

$$\alpha CO_2 = 9.174\times10^{-2} - 3.269\times10^{-3}T + 6.364\times10^{-5}T^2 - 5.378\times10^{-7}T^3. \quad (5)$$

The measured values varied inversely with temperature, from 0.0474 mmol l^{-1} mmHg^{-1} at 20˚C to 0.0363mmol l^{-1} mmHg^{-1} at 35˚C. The predicted relationship between αCO_2 and temperature (equation 5) provided an excellent description of the experimentally determined data (Student's *t*-test, P<0.01). αCO_2 data for *L. kempi* differed only slightly (⩽0.0015mmol l^{-1} mmHg^{-1} from published mammalian values (Severinghaus, 1965; Siggaard-Andersen, 1974; Reeves, 1976). Such differences introduce relatively minor errors in calculations of [CO_2] (e.g. less than 0.06mmol l^{-1} at a P_{CO_2} of 40mmHg).

Fig. 1B gives the measured values of pKa together with the predicted values computed from the following adaptation of Heisler's generalized equation:

$$\text{pKa} = 6.398 \times 1.341\times10^{-2}T + 2.282\times10^{-4}T^2 - 1.516\times10^{-6}T^3 - \log(1.011 + 10^{\text{pH}+0.011T-10.241} + 10^{\text{pH}+0.001T-8.889}). \quad (6)$$

The measured pKa valsues decreased as either temperature or pH increased. The appropriate adaptation of Heisler's equation (equation 6) provided a good description of the measured data (Student's *t*-test, P<0.05). The pKa values for *L. kempi* differed markedly from published mammalian values, not only in absolute value but also in sensitivity to temperature and pH (Table 1). The differences in pKa are sufficiently large to confound analyses of the effects of temperature or pH on turtle plasma [HCO_3^-]. Consequently, the use of mammalian-derived αCO_2 and pKa values cannot be recommended for analyses of the acid–base status of sea turtles.

Table 1. *Comparison of pKa values for* Lepidochelys kempi *(see text) with the mammalian-derived values of Severinghaus (1965), Siggaard-Andersen (1974) and Reeves (1976)*

Temperature (°C)	pH	pKa This study	pKa Severinghaus	pKa Siggaard-Andersen	pKa Reeves
20	7.1	6.197	6.191	6.193	6.184
	7.4	6.189	6.171	6.183	6.184
	7.7	6.174	6.147	6.162	6.184
30	7.1	6.148	6.144	6.138	6.127
	7.4	6.140	6.129	6.128	6.127
	7.7	6.124	6.109	6.108	6.127

The present study demonstrates that Heisler's generalized equations for αCO_2 and pKa (equations 3 and 4) are applicable for use with reptile blood. The appropriate adaptations of Heisler's equations (equations 5 and 6) provided good descriptions of the experimentally determined data for *L. kempi*. Moreover, equation 6 also provided a good description of the pKa values measured by Nicol *et al.* (1983) for the freshwater turtle *Chrysemys picta* at 10 and 20˚C, although not at 30˚C. We conclude that Heisler's (1984) generalized equations provide better estimates of the αCO_2 and pKa of reptile blood than do classical mammalian-derived values.

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References

CAMERON, J. N. (1971). Rapid method for determination of total carbon dioxide in small blood samples. *J. appl. Physiol.* **31**, 632–634.

HEISLER, N. (1984). Acid–base regulation in fishes. In *Fish Physiology*, vol. X (ed. W. S. Hoar and D. J. Randall), pp. 315–401. New York: Academic Press.

HEISLER, N. (1986). Buffering and transmembrane ion transfer processes. In *Acid–Base Regulation in Animals* (ed. N. Heisler), pp. 3–47. Amsterdam: Elsevier Science Publishers.

NICOL, S. C., GLASS, M. L. AND HEISLER, N. (1983). Comparison of directly determined and calculated plasma bicarbonate concentration in the turtle *Chrysemys picta belli* at different temperatures. *J. exp. Biol.* **107**, 521–525.

REEVES, R. B.(1976). Temperature-induced changes in blood acid–base: pH and P_{CO_2} in a binary buffer. *J. appl. Physiol.* **40**, 752–761.

SEVERINGHAUS, J. W. (1965). Blood gas concentrations. In *Handbook of Physiology, Respiration* (ed. W. O. Fenn and H. Rahn), pp. 1475–1487. Washington, DC: American Physiological Society.

SIGGAARD-ANDERSEN, O.(1974). *The Acid–Base Status of the Blood*. Baltimore: William & Wilkins.