J. exp. Biol. **114**, 37–51 (1985)
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EFFECTS OF BODY TEMPERATURE ON RESPIRATION, BLOOD GASES AND ACID-BASE STATUS IN THE TURTLE *CHRYSEMYS PICTA BELLII*

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Accepted 17 October 1983

SUMMARY

Freshwater turtles (*Chrysemys picta bellii* Gray) were acclimated to temperatures of 5, 10, 20 and 30°C for at least 12 days, and pulmonary ventilation, oxygen uptake and arterial pH, P_{CO_2} and P_{O_2} were determined in completely unrestrained specimens. Oxygen uptake ($\dot{V}_{O_2}$) increased overproportionately (6·7-fold) as compared to pulmonary ventilation ($\dot{V}_I$, 4·4-fold) when the temperature increased from 10 to 30°C. The observed rise in arterial P_{CO_2} from 13 (5°C) to 32 mmHg (30°C) was the result of a decrease in $\dot{V}_I/\dot{V}_{O_2}$, whereas an increase of arterial P_{O_2} from 12 Torr at 5°C to about 60 Torr at 20 and 30°C mainly resulted from the effects of intracardiac blood shunting combined with temperature-dependent shifts of the oxygen dissociation curve. Arterial pH fell with rising temperature significantly less ($\Delta pH/\Delta t = -0{\cdot}010$ U/°C) than required for constant relative alkalinity and for constant dissociation of imidazole. The changes of cerebrospinal fluid pH with temperature, calculated from the mean arterial P_{CO_2} values, were even smaller [$\Delta pH/\Delta t_{CSF} = -0{\cdot}008$). It is concluded that the observed temperature dependence of the acid-base status is not in agreement with the alphastat hypothesis.

INTRODUCTION

Turtles encounter a wide range of environmental temperatures throughout the year and have accordingly often been used as model animals for studies concerning the effects of changes in body temperature on the acid-base regulation of ectothermic vertebrates (cf. Jackson, 1978). It was generally found that arterial plasma pH decreased with increasing temperature, the extent of decrease ($\Delta pH/\Delta t$), however, was rather inconsistent, varying by a factor of two even when reported for the same species (cf. Jackson, Palmer & Meadow, 1974; Malan, Wilson & Reeves, 1976). Temperature-dependent regulation of pulmonary ventilation, and thus adjustment of arterial P_{CO_2}, has been suggested as the mechanism responsible for the regulation of pH with changes of temperature. Pulmonary ventilation as a function of temperature has been measured in several studies, but the results vary

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Key words: Acid-base status, alphastat, blood gases, reptilia, respiration, temperature, turtles.

largely between the two extremes of no changes of pulmonary ventilation, and increases in ventilation by a factor of more than two per 10°C temperature rise ($Q_{10} > 2$) (cf. Glass, Hicks & Riedesel, 1979; Jackson, 1971).

The variability of the data obtained to date may partially result from various methodological problems and the non-resting conditions of the experimental animals. Therefore, the aim of the present study was to determine simultaneously the effect of various temperatures on arterial pH, P_{CO_2} and P_{O_2}, on pulmonary ventilation, and on oxygen uptake in the freshwater turtle *Chrysemys picta bellii*, paying particular attention to the constancy of the experimental conditions and to the achievement of a resting state.

MATERIALS AND METHODS

Specimens of *Chrysemys picta bellii* Gray were kept in captivity in large aquaria (20–30°C) equipped with heat lamps and dry basking areas for several months prior to experimentation. The animals were fed on chopped beef liver, chicken meat and commercially available turtle food pellets. At least seven days before implantation of an arterial catheter into the brachial artery under halothane anaesthesia (for details of the procedure see Glass, Boutilier & Heisler, 1983) the animals were acclimated to temperatures of 5°C (number of animals $N = 8$, average weight $\bar{W} = 572$ g), 10°C ($N = 6$, $\bar{W} = 600$ g), 20°C ($N = 8$, $\bar{W} = 708$ g) and 30°C ($N = 7$, $\bar{W} = 579$ g).

After recovery from surgery, the turtle was introduced into the experimental apparatus, which consisted of an aquarium filled with water thermostatted to the respective temperature and shielded against visual disturbances. The animals had no access to the air except in a breathing funnel (for details see Glass *et al.* 1983). This cone-shaped chamber above the water surface was flushed with a constant flow of air (50–250 ml min^{-1}, depending on temperature and size of the turtle). Deviations from the basal flow rate through the breathing funnel represented pulmonary ventilation and were monitored at the outflow by pneumotachography (Model 17212, Godart Statham, Bilthoven, Netherlands). Oxygen consumption was determined from the integrated flow rate through the breathing funnel and the difference in fractional oxygen concentration between inflowing and outflowing gas measured by means of a differential oxygen analyser (Model 5-3A, Applied Electrochemistry Inc., Sunnyvale, CA, U.S.A.).

Pulmonary ventilation and oxygen uptake were monitored for at least 5 days, and blood sampling was not started before the normal periodic breathing pattern and resting O_2 uptake rate were re-established. Then pulmonary ventilation, length of ventilatory and non-ventilatory periods, frequency of ventilation and oxygen consumption were determined (at 10, 20 and 30°C). Arterial pH, P_{CO_2} and P_{O_2} were measured (at 5, 10, 20 and 30°C) several times (up to 10 times) in each animal during ventilatory and non-ventilatory periods during a time period of 72 h. Care was taken to withdraw the arterial blood samples without disturbance of the animals, which would have elicited changes in the ventilatory pattern.

Plasma bicarbonate concentration was calculated on the basis of the Henderson-Hasselbalch equation using values for pK_1''' determined on plasma of *Chrysemys*

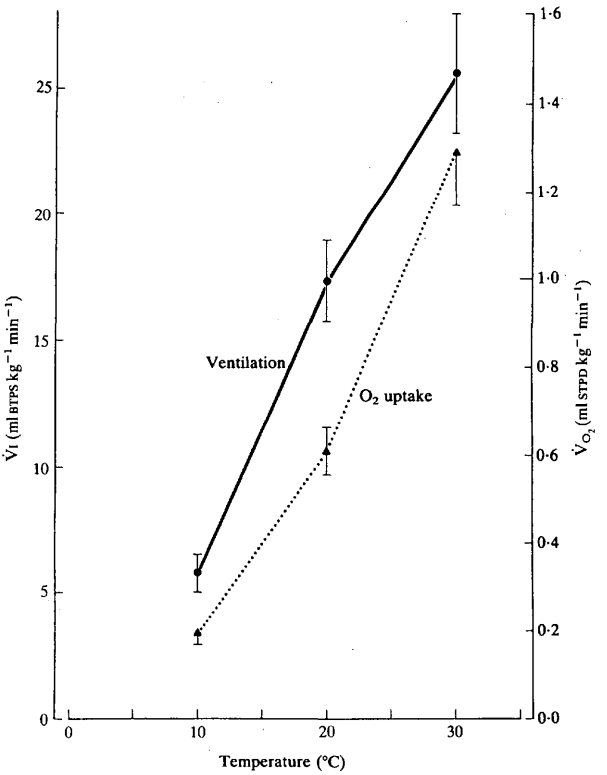

Fig. 1. The effect of body temperature on pulmonary ventilation ($\dot{V}_I$) and oxygen uptake ($\dot{V}_{O_2}$) in *Chrysemys picta bellii* ($\bar{x} \pm$ S.E., $N = 14$ at 10°C, $N = 19$ at 20°C and $N = 13$ at 30°C).

picta bellii (Nicol, Glass & Heisler, 1983) and for CO_2 solubility, α_{CO_2}, adapted from Reeves (1976) for the respective temperature.

Calculation of ventilation was based on inspired volumes.

RESULTS

Pulmonary ventilation increased by a factor of 4·4 with a rise in body temperature from 10 to 30°C, but less than the oxygen uptake which increased 6·7-fold (Fig. 1).

The rises in pulmonary ventilation, $\dot{V}_I$, from 10 to 20°C, and from 20 to 30°C were the result of different mechanisms: from 10 to 20°C, tidal volume remained essentially constant (10°C: 12·7 ± 1·7; 20°C: 10·7 ± 1·2 ml BTPS kg^{-1}), and the

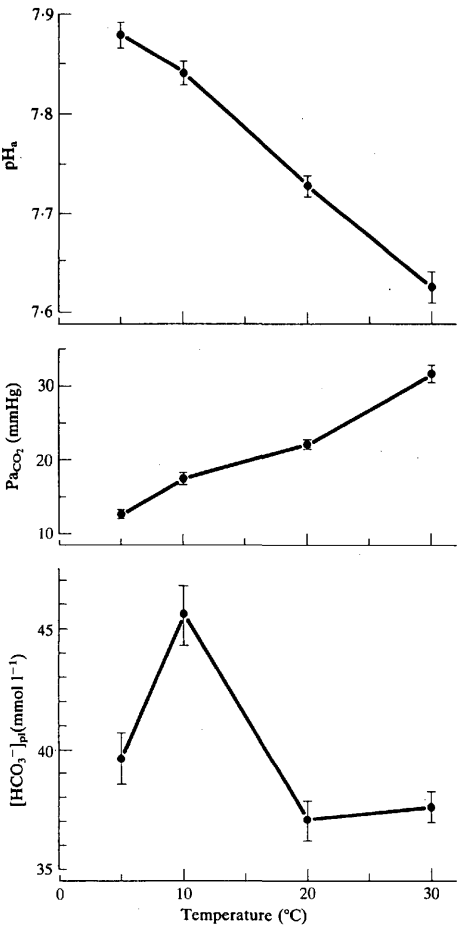

Fig. 2. Relationship between body temperature and arterial plasma pH, P_{CO_2} and bicarbonate concentration ($\bar{x} \pm$ S.E., $N = 60$ at 5°C, $N = 38$ at 10°C, $N = 27$ at 20°C, $N = 19$ at 30°C).

average breathing frequency increased 4-fold (10°C: 0·47 ± 0·05; 20°C: 1·90 ± 0·27 min^{-1}), whereas from 20 to 30°C tidal volume increased almost 2-fold (30°C: 18·3 ± 1·9) and the average breathing frequency fell at 30°C to about 80% of the value at 20°C (30°C: 1·47 ± 0·11). The observed large changes in average breathing frequency were mainly the result of changes in the ratio of the ventilatory period to

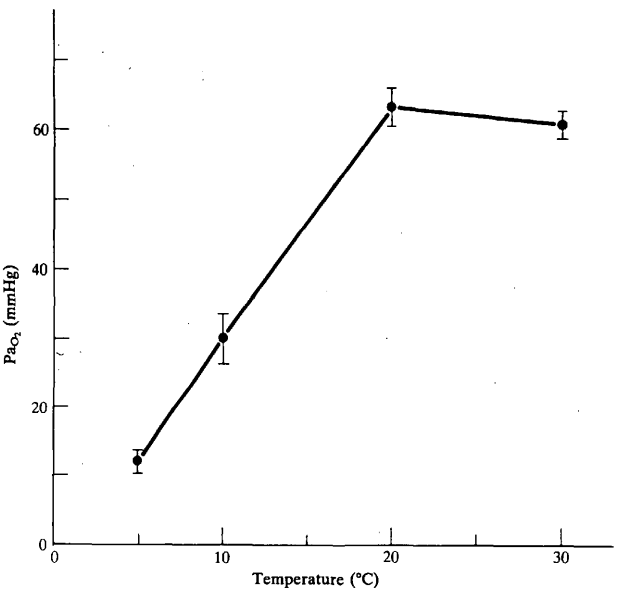

Fig. 3. Correlation between body temperature and arterial P_{O_2} in *Chrysemys* ($\bar{x} \pm$ S.E., N as for Fig. 2).

the length of the total breathing cycle (10°C: 0·031 ± 0·005; 20°C: 0·084 ± 0·011; 30°C: 0·084 ± 0·013); in contrast, the breathing frequency during the ventilatory periods increased with temperature only to a minor extent (10°C: 16·1 ± 2·7; 20°C: 19·6 ± 2·2; 30°C: 21·3 ± 2·4).

Arterial pH and P_{CO_2} changed little during breathing or diving. The slope of the linear regression obtained from all pH measurements during breathing periods at 10, 20 and 30°C [$pH = -0{\cdot}011t_B + 7{\cdot}958$; t_B, body temperature (°C), $N = 41$, $r = 0{\cdot}82$] was identical with that of the regression for diving periods ($pH = -0{\cdot}011t_B + 7{\cdot}939$, $N = 43$, $r = 0{\cdot}82$). Based on all measurements during breathing as well as diving periods, the change in pH was less between 5 and 10°C ($\Delta pH/\Delta t = -0{\cdot}008$ U/°C) than between 10 and 20°C ($\Delta pH/\Delta t = -0{\cdot}011$) and between 20 and 30°C ($\Delta pH/\Delta t = -0{\cdot}010$).

The change in pH with temperature (Fig. 2, upper panel) resulted from changes of both P_{CO_2} and plasma bicarbonate concentration. Arterial P_{CO_2} increased over the whole temperature range from about 13 mmHg at 5°C to about 32 mmHg at 30°C (Fig. 2, middle panel), whereas plasma bicarbonate increased with rising temperature from 5 to 10°C, but then decreased again at 20 and 30°C.

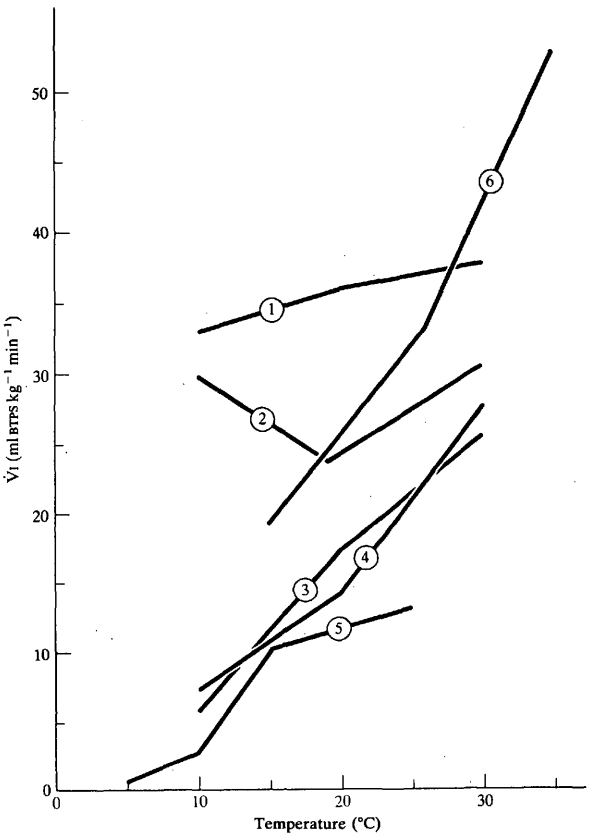

Fig. 4. Comparison of literature data on the relationship between body temperature and pulmonary ventilation ($\dot{V}_I$): 1, *Pseudemys scripta* (Hitzig, 1982); 2, *Pseudemys scripta* (Jackson, Palmer & Meadow, 1974); 3, *Chrysemys picta bellii* (present study); 4, *Pseudemys floridana* (Kinney, Matsuura & White, 1977); 5, *Terrapene ornata* (Glass, Hicks & Riedesel, 1979); 6, *Chelonia mydas* (Kraus & Jackson, 1980).

Arterial P_{O_2} increased linearly from 5 to 20°C (12 to 64 mmHg), but remained essentially constant between 20 and 30°C (Fig. 3).

DISCUSSION

Regulation of ventilation with changes in temperature in turtles is still a matter of controversy. Jackson (1971) and Hitzig (1982) have reported that pulmonary

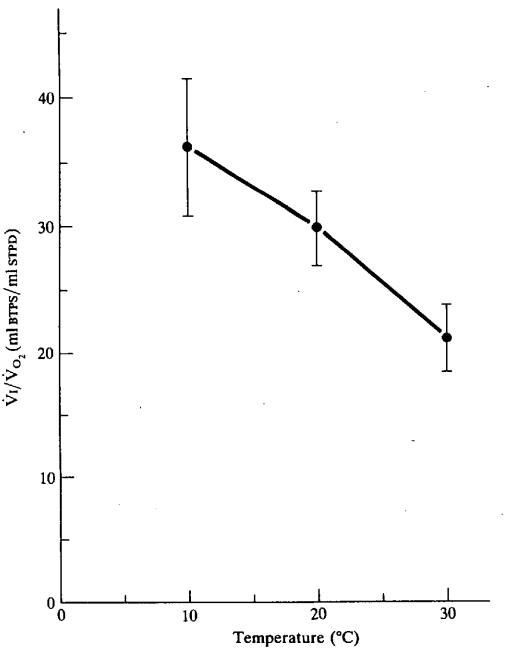

Fig. 5. Ratio of pulmonary ventilation over oxygen uptake ($\dot{V}_I/\dot{V}_{O_2}$) as a function of body temperature in *Chrysemys* ($\bar{x} \pm$ S.E., N as for Fig. 1).

ventilation is maintained virtually unchanged in the freshwater turtle *Pseudemys scripta* over a wide range of temperatures (Fig. 4). In contrast, studies on the closely related species *Pseudemys floridana* (Kinney, Matsuura & White, 1977) and *Chrysemys picta bellii* (present study), as well as on *Terrapene ornata* (Glass *et al.* 1979) and *Chelonia mydas* (Kraus & Jackson, 1980), closely agree in reporting considerable increases of pulmonary ventilation with rising temperature (Fig. 4). However, even when ventilation increases with temperature, this rise is not large enough to maintain a constant ratio of pulmonary ventilation to pulmonary oxygen uptake, $\dot{V}_I/\dot{V}_{O_2}$ (often called 'air convection requirement'). This is illustrated for *Chrysemys picta bellii* in Fig. 5, based on data obtained in the course of the present study. The ratio of $\dot{V}_I/\dot{V}_{O_2}$ has been extensively studied in the turtle *Pseudemys scripta* and the effects of changes in body temperature on arterial P_{CO_2} and pH have been related to the temperature-dependent changes of $\dot{V}_I/\dot{V}_{O_2}$ (Jackson, 1971, 1978).

Alveolar P_{CO_2} (P_{ACO_2}) and $\dot{V}_I/\dot{V}_{O_2}$ are inversely related (cf. Jackson, 1978) and

temperature-dependent changes in $\dot{V}_I/\dot{V}_{O_2}$ account for the rise in arterial P_{CO_2} with increasing temperature in *Chrysemys*.

The fall of arterial P_{O_2} at temperatures below 20°C, however, remains unexplained on this basis. In fact, an increase in $\dot{V}_I/\dot{V}_{O_2}$ with falling temperature, as observed in our experiments below 20°C, would be expected to result in an elevated $P_{A_{O_2}}$, because:

$$P_{A_{O_2}} = P_{I_{O_2}} - (P_{A_{CO_2}}/R_E) + [F], \quad (1)$$

where I denotes inspired, R_E denotes respiratory exchange ratio and [F] represents a relatively small additive correction (cf. West, 1974).

The drastic reduction of arterial P_{O_2} at low temperature (Fig. 3) can only be understood on the basis of intracardiac right-left shunts with the result of large proportions of cardiac blood flow by-passing the pulmonary circulation. A partial arterial desaturation always occurs in turtles, since the intermediate of the three interconnected chambers in the hearts of turtles is common to both pulmonary and systemic circulation, allowing considerable venous admixture to the arterialized blood, similar to the conditions in *Varanus* (Heisler, Neumann & Maloiy, 1983). Arterial P_{O_2} then becomes a function of the mixed systemic venous and the arterialized pulmonary venous blood oxygen content, the shunted blood fraction, and the position of the O_2 dissociation curve of the blood. The right shift of the O_2 dissociation curve with increased temperature (Glass *et al.* 1983) then causes an increase of $P_{a_{O_2}}$ as has been recently pointed out by Wood (1983). Above 20°C, the oxygen loading in the lungs is reduced and the oxygen content of the systemic return falls, resulting in the observed plateau of P_{O_2}. As a result of these mechanisms, the changes of arterial P_{O_2} with temperature in turtles have to be considered to be primarily a function of central vascular shunting events rather than being determined by the ratio of $\dot{V}_I/\dot{V}_{O_2}$, whereas arterial P_{CO_2} is little affected by these factors because of the different characteristics of the CO_2 dissociation curve.

The rise in arterial P_{CO_2} induced by the falling ratio of $\dot{V}_I/\dot{V}_{O_2}$ with increasing temperature is at least partially responsible for the negative correlation between arterial pH and temperature ($\Delta pH/\Delta t = -0{\cdot}008$ to $-0{\cdot}011$ U/°C) in *Chrysemys*. Negative $\Delta pH/\Delta t$ values have been found in most studied ectothermal vertebrates and it has been suggested (Rahn, 1967) that a constant relative alkalinity of arterial blood plasma is maintained instead of a constant arterial pH. The relative alkalinity is defined as the ratio $[OH^-]/[H^+]$ and is related to the actual pH as

$$[OH^-]/[H^+] = 10^{2(pH-pN)}, \quad (2)$$

where pN = pH of neutral water. Maintenance of constant relative alkalinity requires a fall of arterial pH with increase of body temperature, since pN decreases by $-0{\cdot}015$ U/°C at about 35°C to $-0{\cdot}020$ U/°C at about 5°C (*Handbook of Chemistry and Physics*, 56th edn).

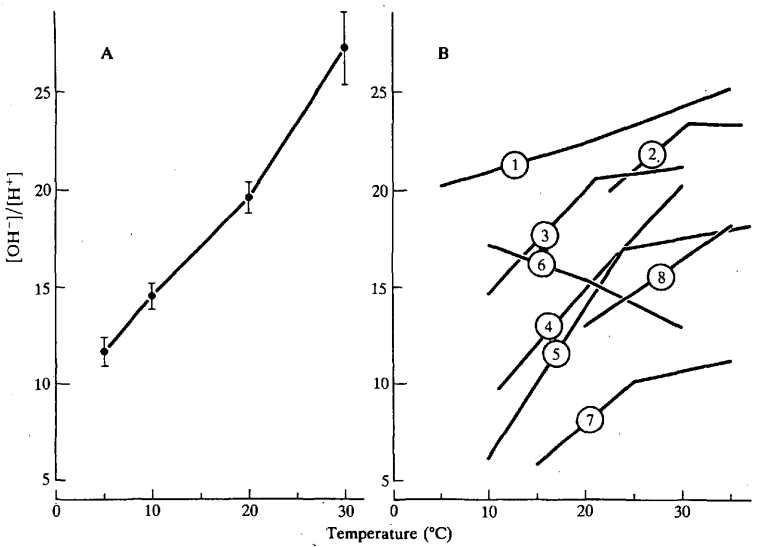

Fig. 6. Relative alkalinity ([OH]/[H⁺]) in the blood of turtles as a function of temperature. (A) *Chrysemys picta bellii* (present study; $\bar{x} \pm$ S.E., N as for Fig. 2). (B) 1, *Chelydra serpentina* (Howell, Baumgardner, Bondi & Rahn, 1970); 2, *Pseudemys floridana* (Kinney, Matsuura & White, 1977); 3, *Pseudemys scripta* (Hitzig, 1982); 4, *Pseudemys scripta* (Jackson, Palmer & Meadow, 1974); 5, *Pseudemys scripta* (Robin, 1962); 6, *Pseudemys scripta* (Malan, Wilson & Reeves, 1976); 7, *Chelonia mydas* (Kraus & Jackson, 1980); 8, *Malacochersus tornieri* (Wood *et al.* 1978).

Values for relative alkalinity of plasma in *Chrysemys* presented in Fig. 6A clearly show that a constant relative alkalinity is not maintained in this species. The deviation is highly significant, and the data compiled in Fig. 6B from the other turtle species studied to date indicate that constant relative alkalinity is rarely achieved, except for some species in the range of higher temperatures. This may be partially due to the above mentioned fact that the neutral pH of water (pN) changes less with temperature at higher than at lower temperatures.

An alternative hypothesis for temperature-dependent acid-base regulation has been provided by Reeves (1972). His imidazole alphastat hypothesis claims that ventilation, and thus P_{CO_2}, is regulated in such a manner that the fractional dissociation of peptide-linked histidine imidazole is kept constant. Alphastat regulation thus implies that $\Delta pH/\Delta t$ in the arterial blood is regulated to equal the value for $\Delta pK/\Delta t$ of the imidazole buffer system. It also implies that, since the histidine imidazole of haemoglobin and plasma proteins represents by far the most important non-bicarbonate buffer system of the extracellular space, the bicarbonate concentration of the compartment is kept constant, especially when transmembrane and transepithelial acid-base relevant ion transfer is excluded (Reeves & Malan, 1976).

Table 1. *Average changes of pH in plasma (pH_{pl}) and cerebrospinal fluid (pH_{CSF}) with changes in body temperature in various species of turtles*

Species	Temperature range (°C)	$\Delta pH_{pl}/\Delta t$	$\Delta pH_{CSF}/\Delta t$ (calculated)	Method of blood sampling	Reference
Pseudemys scripta	10–37	−0·008	−0·011	HP	Robin, 1962
Pseudemys scripta	5–35	—	−0·005	HP	Frankel, Steinberg & Gordon, 1966
Chelydra serpentina	5–20	−0·016	−0·013	HP	Howell, Baumgardner, Bondi & Rahn, 1970
Pseudemys scripta	10–30	−0·009	−0·011	Cath.	Jackson, Palmer & Meadow, 1974
Pseudemys scripta	20–30	−0·013	−0·013	Cath.	Jackson & Kagen, 1976
Pseudemys scripta	5–32	−0·021	—	HP	Malan, Wilson & Reeves, 1976
Pseudemys floridana	22–36	−0·013	−0·008	Cath.	Kinney, Matsuura & White, 1977
Malacochersus tornieri	20–35	−0·011	−0·013	HP	Wood *et al.* 1978
Chelonia mydas	15–35	−0·009	−0·006	Cath.	Kraus & Jackson, 1980
Pseudemys scripta	10–30	−0.013	−0.015*	Cath.	Hitzig, 1982
Chrysemys picta	10–30	−0·011	−0·011	Cath.	Nicol, Glass & Heisler, 1983
Chrysemys picta	5–30	−0.010	−0·009	Cath.	Present study
Average (all studies)	11·4–31·7	−0·012	−0·010		

$\Delta pH_{CSF}/\Delta t$ calculated from arterial P_{CO_2}; ionic transfer between CSF and other body compartments excluded, see text.
* Measured.
Method of blood sampling: Cath. = *via* indwelling arterial catheter, HP = by heart puncture.

The pK value of imidazole changes with temperature ($\Delta pK/\Delta t$) by $-0{\cdot}018$ to $-0{\cdot}024$ U/°C (25°C), depending on ligands and steric arrangement (Edsall & Wyman, 1958). The value for $\Delta pH/\Delta t$ determined for *Chrysemys* in the course of this study in the temperature range of 10–30°C ($-0{\cdot}011$) is only about 60% of the lower limit of the above range and significantly different at a high level ($P < 0{\cdot}001$). This difference is even more pronounced between 5 and 10°C ($\Delta pH/\Delta T = -0{\cdot}008$).

Comparison of the literature data available on $\Delta pH/\Delta t$ in turtle blood reveals that nine out of ten studies report values lower than required for constant imidazole dissociation (Table 1). The average of all studies yields a $\Delta pH/\Delta t$ value of $-0{\cdot}012$, a value very close to the $\Delta pH/\Delta t$ typically found in water-breathing fish (see Heisler, 1980, 1984*a*,*b*). These values do not support the concept of imidazole alphastat regulation. Is this disagreement caused by directional errors introduced by the experimental approach? It turns out that most conceivable sources of error tend to increase $\Delta pH/\Delta t$ rather than reduce it.

(1) If body temperature is measured with a thermometer in the body cavity after killing the animals and taking blood and tissue samples (e.g. Malan *et al.* 1976), then the temperature can only have changed towards room temperature, i.e. animals acclimated to lower temperatures are warmed and *vice versa*. This would result in an underestimate of the real temperature difference between experimental groups and accordingly to an overestimate in $\Delta pH/\Delta t$.

(2) Underestimates of the real temperature range could also result from the temperature difference of 1–3°C between body cavity and environment, which is found in many reptiles (N. Heisler, P. Neumann, H. Weitz & A. Weitz, unpublished data on *Tupinambis nigropunctatus, Varanus exanthematicus, Testudo horsfieldi*). This difference is expected to increase at higher temperatures due to higher metabolism. The resulting underestimate of the temperature difference consequently also increases the $\Delta pH/\Delta t$ value.

(3) Deviations from the normal ventilatory pattern as a result of disturbances prior to blood sampling or due to non-resting conditions may also interfere. From our experience, disturbances of turtles result in a considerable hyperventilation at low temperatures (5–15°C). This would cause a rise in pH and thus also an increase of the $\Delta pH/\Delta t$ value.

(4) The blood sampling procedure may considerably influence the pH pattern. If lactic acid formation occurs as a result of struggling before sampling, then H^+ ions would be extruded from the intracellular space at an extremely high rate (Benadé & Heisler, 1978; Holeton & Heisler, 1983; Holeton, Neumann & Heisler, 1983), lowering blood pH. This effect is least important at lower temperatures because of metabolic pathway limitations and slow H^+ extrusion kinetics, but may result in considerable acidification at higher temperatures, increasing the $\Delta pH/\Delta t$ value. This effect on arterial plasma pH values in fish is well documented (cf. Ali *et al.* 1980; Holeton *et al.* 1983; Holeton & Heisler, 1983). It is probably not fortuitous that the highest $\Delta pH/\Delta t$ values in turtles ($-0{\cdot}021$ and $-0{\cdot}016$: Table 1) have been obtained from measurements in blood sampled by heart puncture.

The evaluation of directional error sources suggests that the average $\Delta pH/\Delta t$ of about $-0{\cdot}012$ represents an upper rather than a lower limit for this parameter.

Evidently alphastat regulation is not typical of the extracellular compartment of turtles. This does not exclude the possibility that alphastat regulation may be achieved in certain intracellular compartments. In fact, the only study available on the relationships of intracellular pH and temperature in turtles (Malan *et al.* 1976) reports $\Delta pH/\Delta t$ values in the intracellular space of white muscle (−0·0186 U/°C) and liver (−0·0233 U/°C) in the range of the $\Delta pK/\Delta t$ values of imidazole compounds. These data, however, have been obtained in the study with the highest extracellular $\Delta pH/\Delta t$ value (−0·021 U/°C), which is about 170 % of the average value determined in turtles. If the extracellular $\Delta pH/\Delta t$ value was in fact affected by the sources of error discussed above, then the intracellular $\Delta pH/\Delta t$ values would have been overestimated by an even larger factor because of the properties of the DMO method for measurement of intracellular pH (see Appendix).

Regardless of these considerations, the change in P_{CO_2} observed in the present study for *Chrysemys* is far too small to cause a change of plasma pH with temperature in parallel with the changes in pK_{im} (for model calculations see Heisler, 1978, 1984*a*,*b*; Heisler & Neumann, 1980).

In intracellular compartments, the $\Delta pH/\Delta t$ value is less influenced by the change in P_{CO_2} than by the ratio of imidazole-like ($\Delta pK_{im}/\Delta t \sim -0{\cdot}020$ U/°C) to phosphate-like ($\Delta pK_{ph}/\Delta t \sim -0{\cdot}002$ U/°C) non-bicarbonate buffer values (β_{im}/β_{ph}) (Heisler & Neumann, 1980; Heisler, 1984*a*,*b*). In the extracellular space this ratio is in the range of 10–30, whereas the intracellular ratio has been estimated (Reeves & Malan, 1976) and determined (Heisler & Neumann, 1980; Heisler, 1984*a*,*b*) to be in the range of 1 to 4 in various tissues. Intracellular bicarbonate concentrations are lower by factors of 2 to 5, according to the lower pH values than those in extracellular compartments. In addition, intracellular non-bicarbonate buffer values are expected to be much higher on the basis of data from other vertebrate species (Heisler & Piiper, 1971, 1972; Heisler & Neumann, 1980; Heisler, 1984*a*). As a consequence of these conditions, intracellular $\Delta pH/\Delta t$ values are rather independent of P_{CO_2} (see Heisler, 1984*a*,*b*). If $\Delta pH/\Delta t$ in any intracellular compartment is regulated to a value different from that predetermined by the buffer values ratio (β_{im}/β_{ph}), this has to be performed mainly by transmembrane transfer of acid-base relevant ions (i.e. HCO_3^-, OH^-, or H^+ in the opposite direction). Consequently, an overall imidazole alphastat acid-base regulation in all body compartments by adjustment of only pulmonary ventilation, as propounded by Reeves (1972) and Reeves & Malan (1976), cannot be expected.

Histidine-imidazole protein residues as sensors for the regulation of ventilation *per se* would not be positioned optimally in an intracellular compartment. Minor non-respiratory disturbances of pH would result in extreme changes in ventilation, which would nevertheless not be sufficient to maintain the imidazole dissociation due to the steep slope of the intracellular CO_2-buffer curve. In contrast, the cerebrospinal fluid (CSF), which is claimed to be the environment for respiratory receptors in higher vertebrates, contains virtually no non-bicarbonate buffers, except for a small concentration of phosphates, and is known to have extremely close diffusive contact with the arterial blood. Accordingly, it would be an optimal site for an alphastat sensor and in fact Hitzig (1982) has reported that $\Delta pH/\Delta t$ in the CSF of *Pseudemys* was −0·015 U/°C, not significantly different from the lower limit of −0·018 U/°C for $\Delta pK/\Delta t$ of physiological imidazole compounds.

Data on CSF-pH are not available from the other studies on turtles cited above. But since P_{CO_2} in arterial blood and CSF are little different from each other in higher vertebrates and also in turtles (e.g. Hitzig, 1982), $\Delta pH/\Delta t$ of the CSF can be estimated. If the Henderson-Hasselbalch equation is applied twice to values at different temperatures [indices (1) and (2)] and the two equations are subtracted, then

$$\Delta pH = pK'_1 + \log \frac{[HCO_3^-]_{(1)}}{[HCO_3^-]_{(2)}} + \log \frac{\alpha_{(2)}}{\alpha_{(1)}} + \log \frac{P_{CO_2,(2)}}{P_{CO_2,(1)}} . \quad (3)$$

If, as has been claimed, no ionic transfer occurs between CSF and other compartments, then the bicarbonate concentration is constant, since CSF is virtually free of non-bicarbonate buffers:

$$\Delta pH = pK'_1 + \log \frac{\alpha_{(2)}}{\alpha_{(1)}} + \log \frac{P_{CO_2,(2)}}{P_{CO_2,(1)}} . \quad (4)$$

Assuming that P_{CO_2} in CSF is the same as in plasma the change in pH with changes in temperature can be calculated on the basis of pK'_1'' and α_{CO_2} values for cerebrospinal fluid (Siggaard-Andersen, 1974). The P_{CO_2} data of this study on *Chrysemys* result then in an estimate of $\Delta pH/\Delta t$ in the CSF of $-0{\cdot}009$ U/°C, a value significantly lower than the lower limit of $\Delta pK_{im}/\Delta t$ of $-0{\cdot}018$ U/°C, and thus do not confirm the alphastat regulation claimed for *Pseudemys* (Hitzig, 1982). Also all literature data (Table 1) lead to $\Delta pH/\Delta t$ values lower than required for alphastat regulation and there is no example of a value even approaching the lower limit of $\Delta pK_{im}/\Delta t$.

In addition, the model condition of excluded ionic transfer between CSF and other compartments is a most unlikely condition *in vivo*. The ion transfer mechanisms which have been shown to exist for the structures surrounding the CSF in higher vertebrates (e.g. Ahmad & Loeschke, 1982*a*,*b*) probably also exist in lower vertebrates and interfere with the changes in pH induced by the temperature-dependent changes in P_{CO_2}. If constant imidazole dissociation is achieved by means of ionic transfer, this must clearly diminish the role of this parameter for the regulation of pulmonary ventilation. Accordingly, the adjustment of ventilation very probably follows other criteria.

It can be concluded that the rise in arterial P_{CO_2} with increasing temperature in *Chrysemys* is a result of readjustment of the ratio $\dot{V}_I/\dot{V}_{O_2}$, whereas the temperature-dependent alterations of arterial P_{O_2} have to be mainly attributed to the interrelationship between the right shift of the oxygen dissociation curve with increased temperature and the partial desaturation of arterial blood as a result of central vascular R-L shunting. The observed temperature-dependent fall in plasma pH, which is considerably and significantly smaller than required for constant pK_{im}, is, however, only partially due to the rise in P_{CO_2} with increasing temperature, but is also effected by changes in plasma bicarbonate. Analysis of the changes in P_{CO_2} with variation of temperature in various species of turtles suggests that, if constant dissociation of histidine-imidazole is ever achieved in any body compartment, closed-system buffering is less important for this type of acid-base regulation than transmembrane and transepithelial transfer of acid-base relevant ions.

APPENDIX

The intracellular pH can be determined from the distribution of 5,5-dimethyl-2,4-oxazolidinedione (DMO) between intracellular and extracellular space of a tissue (Waddell & Butler, 1959) according to the following formula (Heisler & Piiper, 1972):

$$pH_i = pK'_{DMO} + \log\left[\frac{C_i}{C_e}(10^{pH_e - pK'_{DMO}} + 1) - 1\right],$$

where C is the concentration of DMO, indices 'i' and 'e' denote intracellular and extracellular. If the extracellular pH is misestimated by the measurement, or is changed *in vivo* shortly before the measurement by hyperventilation, production of lactic acid or other mechanisms, then the slow distribution of DMO across the cell membranes cannot follow the quick changes in the pH difference between intracellular and extracellular compartment. Accordingly, the concentration ratio C_i/C_e is the same as during the preceding steady state. The steady state intracellular pH is then misestimated not only to the same extent as the extracellular pH, but according to the above formula more than pH_e by a factor depending on the absolute pH range, and the difference between pH_e and pH_i. This factor is between 1·16 and 1·07 for the pH_e and pH_i values reported for various tissues of turtles at 30°C (Malan *et al.* 1976).

The authors gratefully acknowledge the skilful technical assistance of Mrs S. Glage and Mr G. Forcht. Supported by Alexander von Humboldt Stiftung and Deutsche Forschungsgemeinschaft and the Danish National Science Research Council.

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