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SHORT COMMUNICATION

THE EFFECT OF SEASONAL ACCLIMATIZATION ON THE BUFFERING CAPACITY AND LACTATE DEHYDROGENASE ACTIVITY IN TISSUES OF THE FRESHWATER TURTLE *CHRYSEMYS PICTA MARGINATA*

BY JOHN M. OLSON AND KENNETH M. CRAWFORD
Department of Biology, The University of Michigan, Ann Arbor, Michigan 48109, USA

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Freshwater turtles are remarkably tolerant of anoxic conditions, a tolerance essential for animals that spend much of their time submerged (Belkin, 1963). Many metabolic adaptations for efficient anaerobiosis have been elucidated in turtles, and are essential for allowing these animals not only to dive over a wide range of water temperatures (Gatten, 1981) but also to survive the long hibernation during winter in the higher latitudes (see Penney, 1987, for a review).

Because of the importance of pH to the charge-state and consequently the function of several metabolic proteins, acid–base balance has been studied extensively in freshwater turtles (Reeves, 1977). Extracellular buffering is achieved primarily through ventilatory control of P_{CO_2} and bicarbonate concentrations (Jackson & Silverblatt, 1974) and, in long-term anoxia, through adjustments in plasma ion concentrations that maintain the strong ion difference (Stewart, 1978) and buffer the lactate produced (Jackson & Heisler, 1982, 1983, 1984).

Although measurements of intratissue acid–base status have been made in turtles (Malan *et al.* 1976), including measurements during anoxia (Clark & Miller, 1973; Jackson & Heisler, 1983), the contribution of intratissue, non-bicarbonate buffering has largely been neglected. We therefore measured the intratissue buffering capacities of the heart and pectoralis muscles of freshwater turtles, tissues that often depend upon anaerobiosis (Reeves, 1963) and are important in circulation and locomotion during submergence. We also determined if intratissue buffering capacities and lactate dehydrogenase activities change seasonally. Because turtles survive for longer periods while submerged at cold temperatures and depend predominantly upon anaerobic glycolysis for energy during all dives under these thermal conditions (Gatten, 1981), intratissue buffering capacities may be higher in tissues from spring- and autumn-acclimatized turtles than in those from summer-acclimatized animals. In addition to the three seasons above,

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tissues from winter-acclimatized animals were analyzed to assess the capacity for intratissue buffering during hibernation. The term acclimatization, as used here, refers to the fact that these turtles experience many seasonally fluctuating parameters in addition to temperature.

Twenty naturally acclimatized painted turtles (*Chrysemys picta marginata*) were collected near Ann Arbor, Michigan, in basking or baited traps during April 1986, July 1985 and October–November 1985 ('spring', 'summer', and 'autumn' samples, respectively) or from under the ice in January 1987 ('winter' sample; see Crawford, 1988). Mean water temperatures at the time of capture varied considerably with season (3·5 °C in winter, 14–15 °C in spring and autumn and 26·9 °C in summer); however, no significant differences in the size of the animals (mean mass or plastron length) existed among the four seasonal samples ($P > 0{\cdot}10$; ANOVA). Animals were transported back to the laboratory and killed by cervical dislocation. The entire heart and pectoral muscles were immediately dissected, weighed (range of fresh masses 0·4–1·2 g and 0·6–2·9 g, respectively), wrapped tightly in aluminum foil, and quick-frozen on dry ice. Wrapped tissues were stored frozen at −70 °C in air-tight vials for no longer than 10 weeks until assayed for buffering capacity, lactate dehydrogenase activity and protein content.

Intratissue buffering capacity was measured following the methods of Castellini & Somero (1981), except that tissues were homogenized in 20 vol of 0·9 % NaCl and the titration was performed at 25 °C. As the titration was linear over the pH range used (pH 6–7), non-bicarbonate buffering capacity (β) was calculated as the number of μmol of base required to increase the equivalent of 1 g of tissue by 1 pH unit (slykes). Lactate dehydrogenase (LDH; E.C.1.1.1.27) activities were measured spectrophotometrically at 340 nm, according to the method of Beenakkers (1969). Assays were performed at 10 and 25 °C in a final volume of 1 ml. The protein content of heart and pectoral muscle was determined using the Lowry method.

A two-way ANOVA with repeated measures was used to test for significant differences among groups. The two major grouping variables – season and tissue – were considered orthogonal, fixed treatment effects.

Intratissue β values of both the heart and pectoralis muscles of *C. picta* were affected by seasonal acclimatization, though the two tissues were affected differently (Fig. 1). β values for the heart from spring-acclimatized animals were the highest measured for this tissue ($P < 0{\cdot}05$); the corresponding values for heart from animals collected in the other three seasons were lower and not statistically different from each other. In contrast, the buffering capacities of the pectoralis muscle were more variable across seasons, with values of winter-acclimatized turtles the lowest measured for this tissue and those of summer- and autumn-acclimatized animals the highest ($P < 0{\cdot}0001$).

Seasonal variation in the intratissue concentrations of protein (Table 1; overall $P < 0{\cdot}001$), glycogen and water (K. M. Crawford, unpublished observations) confounds the analysis of seasonal differences of parameters normalized per gram of wet mass. Comparisons of both β and LDH activity normalized per milligram of

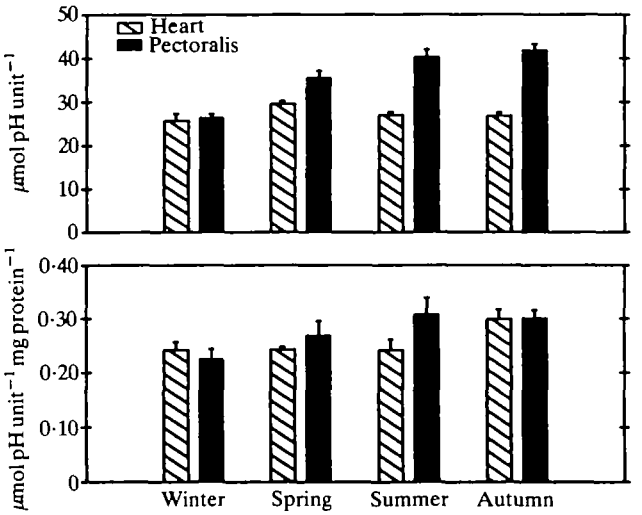

Fig. 1. Intratissue non-bicarbonate buffering capacities (β) of the heart and left pectoralis muscle of *Chrysemys picta*. Bars are means (in μmol pH unit^{-1}, top panel; μmol pH unit^{-1} mg protein^{-1}, bottom panel) and thin bars are 1 S.E.M. ($N = 5$ for each sample).

protein at least partially correct for the sometimes profound seasonal changes in the proportion of metabolically active tissue and, therefore, are more rigorous. Moreover, expressing β per milligram of protein may reveal if qualitative changes in the peptide pool occur seasonally, as only a portion of the peptide pool (primarily histidyl-containing residues; Reeves & Malan, 1976) contributes to buffering in the physiological pH range. Therefore, all subsequent statistical comparisons were made using β values and LDH activities expressed on a per milligram of protein basis.

The seasonal patterns described above generally persisted when buffering capacities were expressed on this basis (Fig. 1, bottom). As was the case when expressed on a per gram of wet mass basis, seasonal variation of β in the heart was much lower than that for the pectoralis muscle. For heart, the highest values were observed in autumn-acclimatized animals ($P < 0{\cdot}05$), whereas the corresponding values in the other three seasons were lower and not statistically different from each other. In addition, β values of the pectoralis muscle of winter-acclimatized animals were the lowest of the four seasonal samples ($P < 0{\cdot}0001$), but those from spring-, summer- and autumn-acclimatized turtles were not statistically different from each other. The buffering capacities of the pectoralis muscle were higher in the seasons in which turtles are active (spring to autumn) than in overwintering animals, possibly reflecting the enhanced glycolytic flux in this tissue during these seasons. The results above indicate, however, that β values of tissues from animals collected in spring and autumn were no higher than those of animals collected in summer.

Table 1. *Protein concentrations and lactate dehydrogenase (LDH) activities*

Sample	Protein ($mg\,g^{-1}$)	LDH activity ($\mu mol\,min^{-1}\,mg\,protein^{-1}$) 10°C	25°C
Winter			
Heart	$106{\cdot}4 \pm 3{\cdot}0^{a,d}$	$1{\cdot}02 \pm 0{\cdot}06^{c}$	$2{\cdot}62 \pm 0{\cdot}27^{c}$
Pectoralis	$119{\cdot}5 \pm 7{\cdot}0$	$0{\cdot}86 \pm 0{\cdot}09^{a}$	$2{\cdot}20 \pm 0{\cdot}23$
Spring			
Heart	$121{\cdot}7 \pm 0{\cdot}8^{c}$	$1{\cdot}23 \pm 0{\cdot}05^{c}$	$2{\cdot}64 \pm 0{\cdot}16^{c}$
Pectoralis	$134{\cdot}8 \pm 7{\cdot}9$	$1{\cdot}11 \pm 0{\cdot}06$	$2{\cdot}85 \pm 0{\cdot}22$
Summer			
Heart	$113{\cdot}7 \pm 6{\cdot}3^{c}$	$1{\cdot}23 \pm 0{\cdot}15^{c}$	$3{\cdot}12 \pm 0{\cdot}29^{b}$
Pectoralis	$134{\cdot}0 \pm 9{\cdot}0$	$0{\cdot}93 \pm 0{\cdot}08$	$2{\cdot}70 \pm 0{\cdot}27$
Autumn			
Heart	$90{\cdot}4 \pm 4{\cdot}4$	$1{\cdot}69 \pm 0{\cdot}08$	$4{\cdot}00 \pm 0{\cdot}16$
Pectoralis	$139{\cdot}3 \pm 3{\cdot}4^{***}$	$1{\cdot}16 \pm 0{\cdot}11^{*}$	$2{\cdot}58 \pm 0{\cdot}20^{**}$

Values are mean ± 1 S.E.M. ($N = 5$ for each sample).

Superscript letters signify significant differences among seasons for a given tissue (ANOVA). a–c, significantly different from corresponding value in the autumn sample (a, $P < 0{\cdot}05$; b, $P < 0{\cdot}01$; c, $P < 0{\cdot}005$); d, significantly different from value for heart in spring ($P < 0{\cdot}05$).

Asterisks signify significant differences between tissues within a season: * $P < 0{\cdot}01$; ** $P < 0{\cdot}005$; *** $P < 0{\cdot}001$).

Seasonal differences in the protein-specific activities of LDH roughly paralleled those seen in buffering capacities (Table 1). As is the case for β, the heart of autumn-acclimatized turtles possessed the highest LDH activity at 10°C ($P < 0{\cdot}005$) and 25°C ($P < 0{\cdot}05$). In addition, the activity of LDH in the pectoralis muscle was lowest in winter ($P < 0{\cdot}05$), and no significant differences existed among the other three seasons. However, despite the similar effect of acclimatization on both β and LDH activities (Fig. 1; Table 1), these two variables were correlated only in the heart ($P < 0{\cdot}05$). The absence of a consistent correlation between LDH activities and β in this intraspecific study differs from the patterns observed for these two measures of glycolytic ability in an interspecific study of fishes (Castellini & Somero, 1981).

The possibility that the two tissues possess different buffering capacities (Jackson & Heisler, 1983) and LDH activities was also examined. Between-tissue comparisons revealed protein-specific β values in the two tissues were not statistically different in any season ($P > 0{\cdot}10$; Fig. 1). Similarly, LDH activities in the heart and pectoralis muscles were similar at both assay temperatures in all seasons except autumn ($P < 0{\cdot}01$; Table 1). The markedly higher LDH activities in the heart of autumn-acclimatized turtles was in part due to the especially low protein content measured (Table 1). Determining why heart in autumn-acclimatized turtles has such a low protein content requires further study; however, it

possibly reflects seasonal and tissue-specific differences in the accumulation of glycogen and accompanying water.

Neither β nor the LDH activity of the heart and pectoralis muscles were high; even by ectothermic vertebrate standards (cf. tissues of fishes in groups I and II of Castellini & Somero, 1981). These values, however, are similar to those found in the skeletal muscles of animals where demands for high glycolytic flux are usually rare or short-lived [e.g. striated muscles of the frog (Reeves & Malan, 1976) and fish species that are sit-and-wait predators (Castellini & Somero, 1981)]. This pattern of catabolic demand is also shared by painted turtles; neither the typical anti-predator responses (retracting head and limbs into the shell or engaging in short, evasive dives) nor the typical foraging mode (scavenging) requires that turtles sustain high levels of activity. These animals appear to rely instead on lower levels of activity which, along with the especially low metabolic rates at cold temperatures, prolong the depletion of glycogen stores and prevent rapid production of lactate in the cell. Moreover, the modest intratissue non-bicarbonate buffering capacities observed here, as well as the modest non-bicarbonate buffering capacities of turtle blood (Ultsch & Jackson, 1982), are augmented by other buffering pools (e.g. plasma, pericardial and peritoneal fluids, bone) in the maintenance of acid–base balance. Adjustments of acid–base status in these pools can be very rapid, especially if turtles have access to air (Jackson & Kagen, 1976).

The generally low intratissue β values, LDH activities and protein concentrations in the tissues of winter-acclimatized turtles (Fig. 1; Table 1) suggest the heart and pectoralis are adapted for low glycolytic flux during this season. This pattern is consistent with the low overall metabolic rates observed in *C. picta* during prolonged submergence in the cold (Herbert & Jackson, 1985) and the reduced activities of key metabolic enzymes in tissues of *C. picta* at low temperatures (e.g. ATPase, Rotermund & Privitera, 1972; phosphofructokinase, Olson, 1987). Low intratissue buffering capacities would further impair the ability to maintain pH balance during prolonged submergence in winter, possibly hastening the metabolic acidosis observed in tissues after 2–4 weeks of submergence at 3°C (Jackson & Heisler, 1983). Such an acidosis could be adaptive by sparing glycogen stores and further reducing metabolic flux *via* a pH-mediated inactivation of metabolic enzymes, as was suggested for phosphofructokinase in mammals (Hand & Somero, 1982).

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