Lactic Acid Buffering by Bone and Shell in Anoxic Softshell and Painted Turtles

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ABSTRACT

We tested two hypotheses: first, that the inferior anoxia tolerance of the softshell turtle, *Apalone spinifera*, compared to the western painted turtle, *Chrysemys picta bellii*, is related to its less mineralized shell, and second, that turtle bone, like its shell, stores lactate during prolonged anoxia. Lactate concentrations of blood, hindlimb bone, and shell were measured on normoxic *Apalone* and *Chrysemys* and after anoxic submergence at 10°C for 2 and 9 d, respectively. Blood and shell concentrations of Ca^{2+}, Mg^{2+}, Na^{+}, K^{+}, and inorganic phosphate (P_i; for shell only) were also measured. Because a preliminary study indicated lactate distribution in *Chrysemys* throughout its skeleton during anoxia at 20°C, we used hindlimb bones as representative skeletal samples. *Apalone* shell, though a similar percentage of body mass as *Chrysemys* shell, had higher water content (76.9% vs. 27.9%) and only 20%–25% as much Ca^{2+}, Mg^{2+}, CO_2, and P_i. When incubated at constant pH of 6.0 or 6.5, *Apalone* shell powder released only 25% as much buffer per gram wet weight as *Chrysemys* shell. In addition, plasma [Ca^{2+}] and [Mg^{2+}] increased less in *Apalone* during anoxia at an equivalent plasma lactate concentration. Lactate concentrations increased in the shell and skeletal bone in both species. Despite less mineralization, *Apalone* shell took up lactate comparably to *Chrysemys*. In conclusion, a weaker compensatory response to lactic acidosis in *Apalone* correlates with lower shell mineralization and buffer release and may partially account for the poorer anoxia tolerance of this species.

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Introduction

Although freshwater turtles in general possess considerable tolerance to anoxia (Belkin 1963), significant differences in this capacity exist among species (Ultsch et al. 1984). These differences may relate to their individual environments and habits and may be explained by varying expression of traits contributing to survival during anoxia.

A particularly striking contrast exists between the painted turtle, *Chrysemys picta*, and the softshell turtle, *Apalone spinifera*. *Chrysemys* is the most anoxia-tolerant turtle yet studied and can recover from submergences in O_2-depleted water lasting 3 mo at 3°C and 10 d at 10°C (Herbert and Jackson 1985*a*) and can remain alive under these conditions for as long as 5 mo and 17 d, respectively (Ultsch et al. 1984). The softshell turtle, in contrast, survived only 2.6 d of anoxia at 10°C (Ultsch et al. 1984), and submergence durations from which it could recover are undoubtedly considerably less. Compared to *Chrysemys*, *Apalone* has a more gas-permeable integument, and by selecting microhabitats with adequate aquatic oxygen levels, this turtle can support aerobic metabolism by oxygen uptake from the water. In aerated water at 10°C, for example, most submerged *Apalone* survived more than 100 d, and their limit was not established (Ultsch et al. 1984). *Chrysemys*, on the other hand, has an integument less suited for aquatic gas exchange, and the advantages it gains from aquatic oxygen are less dramatic. During hibernation it may select refuges in anoxic mud (Ernst 1972), although this is not always the case (St. Clair and Gregory 1990; Crocker et al. 2000).

A key physiological difference between *Chrysemys* and *Apalone* during anoxic submergence at 10°C is the rate at which plasma lactate concentration increases, an index of anaerobic metabolic rate. In *Chrysemys*, lactate increased over the 10 d of submergence by 0.28 mmol L h^{-1}, which was less than 30% the rate of increase in *Apalone* over 1 d of anoxia (Jackson et al. 1984). As a consequence, blood pH fell more rapidly in *Apalone*, and this may have been a major factor limiting survival in this animal. The low rate of lactate accumulation in *Chrysemys* and the associated depressed rate of anaerobiosis (Herbert and Jackson 1985*b*) are fundamental traits possessed by this turtle that permit long anoxic submergences.

Compensatory buffering capability may be another physiological difference between these turtles that helps explain their respective tolerances to anoxia. *Chrysemys* has higher extracellular [HCO_3^-] than *Apalone* (Ultsch et al. 1984), and of even greater importance, *Chrysemys* has a large calcified shell that accounts for the bulk of lactic acid buffering by this animal during long-term anoxic submergence at low temperature (Jackson 1997). The shell functions in two ways: first, calcium and magnesium carbonates are released from the shell to buffer lactic acid in the extracellular fluid, and second, lactic acid enters the shell where it is buffered and sequestered during the anoxic period (Jackson 1997; Jackson et al. 1996). The softshell turtle, *Apalone*, as its name indicates, has a less mineralized shell and, therefore, may lack the capacity for shell buffering possessed by *Chrysemys*. This lack may be a second factor accounting for the poorer performance of *Apalone* during anoxia.

A primary object in this study, therefore, was to quantitatively compare shell composition and function during anoxia in these two chelonian species in order to consider the hypothesis that differences contribute to the disparity in anoxia tolerance. A second related objective was to determine whether the turtle's skeleton (that portion not incorporated into the shell) also functions as a storage site for lactate during anoxia and to compare the behavior of the two species in this regard.

Material and Methods

Animals

Western painted turtles (*Chrysemys picta bellii*) and softshell turtles (*Apalone spinifera aspera*) were obtained from commercial sources in Wisconsin and Alabama, respectively. They were housed before the study under a 10L : 14D photoperiod in large tanks with shallow water and were fed 3–4 times per week with earthworms. The studies were performed in late December and early January.

Experimental Protocols—In Vivo

Each of the projects involved analysis of blood, bone, and shell samples from turtles in either a control condition or following a period of anoxic submergence.

Experiment 1: Chrysemys *at 20°C.* This preliminary procedure was designed to determine if turtle bone, like its shell, accumulates lactate during anoxia and if so, to establish whether this occurs throughout the skeleton. Five turtles were studied at 20°C; two served as controls and three were studied after 6 h submergence without access to air. All turtles were surgically fitted with chronic catheters in the subclavian artery (Jackson et al. 1974) under Brevital anesthesia (10 mg kg^{-1} via the anterior limb pocket).

Blood samples were taken via the catheter and lactate was determined on the plasma fraction. The turtles were then killed with an intravascular overdose of Brevital, and shell samples (0.7-cm diameter disks) were taken using a hand punch (Roger Whitney of Rockford, model 5 Jr) from both the carapace and plastron. These samples were weighed and then stored at –80°C until processing. In earlier studies (Jackson et al. 1996; Jackson 1997), we found that lactate distributes nearly uniformly throughout the shell of this turtle so that individual samples can be regarded as representative of the whole shell. The rest of the carcass was kept cold and the elements of the skeleton not connected to the shell were dissected out, cleaned of adherent tissue, and stored at –80°C until processing. Included were the long bones of the fore- and hindlimbs, the pectoral and pelvic girdle bones, and the skull. Skull was only tested on anoxic turtles. The procedures for powdering the shell and bone samples and analyzing them for lactate are described below.

Experiment 2: Chrysemys *and* Apalone *at 10°C.*

Chrysemys. Two groups of turtles with arterial catheters implanted as above were studied. The first ($N = 3$) were control animals kept at 10°C for 1 d with access to air before being killed with an overdose of Brevital. A blood sample was taken for plasma lactate, and shell samples and hind legs were collected and stored. The long bones of the leg (femur, tibia, and fibula) were later isolated, cleaned, and stored at −80°C with the shell samples. The second group of turtles ($N = 5$) was submerged in N_2-equilibrated water at 10°C for 9 d before undergoing the same terminal sampling procedure. In an earlier study (Herbert and Jackson 1985*a*), we found that painted turtles could fully recover from anoxic submergences of this duration. In the anoxic group, blood and shell (carapace) margin samples were taken at 0, 3, 6, and 9 d. Blood hematocrit was measured, and plasma was analyzed for concentrations of lactate, Na^+, K^+, total Ca^{2+}, and total Mg^{2+}; shell and bone were analyzed for lactate. One of the anoxic turtles lost its catheter early in the submergence period so data were only obtained from four animals.

Apalone. Uncatheterized turtles were studied and all were slowly cooled to 10°C over a period of 4 d and then kept at that temperature for 1 d with access to air before study. The control group ($N = 5$) was studied at this time. The turtles in the experimental group ($N = 4$) were kept in water at 10°C for an additional 2 d but were denied access to the surface, and the water was continuously bubbled with N_2. In an earlier study (Ultsch et al. 1984), we established that 2 d of anoxia was near the tolerable limit for softshell turtles at 10°C. The turtles in each group were killed, the plastron was removed, and blood was sampled by heart puncture. The entire shell, including both plastron and carapace, was thoroughly cleaned of adherent underlying muscle, weighed, and stored at −80°C for later processing. The hind legs were removed and stored similarly for later harvesting and study of the bones. Blood was tested for

hematocrit and plasma was analyzed for the concentrations of lactate, Na^+, K^+, total Ca^{2+}, and total Mg^{2+}.

The reason that the painted turtles were catheterized but the softshell turtles were not was that we wished to obtain intermediate plasma and shell values on painted turtles to compare to the final values obtained from the softshell turtles. Based on earlier studies, responses to anoxic submergence are not affected significantly by catheterization.

Experimental Protocols—In Vitro

Buffering Properties of Shell. Dried shell powder from each of the normoxic *Apalone* was incubated in Ringer's solution containing 50 mM lactate, and the volume of 1 N HCl required to maintain constant pH at either 6.0 or 6.5 was recorded. This procedure was the same as employed previously on *Chrysemys* shell powder (Jackson et al. 1999) and permitted direct comparison of the shell buffering properties of the two species. The setup is described in detail in the previous publication. Briefly, a flask contained 10 mL of solution with the following composition (in mmol L^{-1}): 100 Na^+; 2.5 K^+; 2.0 Ca^{2+}; 1.0 Mg^{2+}; 50 lactate$^-$. To achieve pH values of 6.0 and 6.5, [HCO_3^-] was 0.6 and 2.0 mmol L^{-1}, respectively, and Cl^- provided the remaining negative charge.

The solution was equilibrated with a gas mixture (2.5% CO_2/balance N_2) until stable pH was achieved, and then shell powder was added by rotating an attached bulbous sidearm containing a weighed amount of powder. The solution and powder were continuously mixed with a magnetic stirrer, and solution pH was held constant by titration with 1 N HCl using a pH-stat system (Radiometer model TTT60 Titrator, ABU11 Autoburette, PHM 62 pH meter, and pH electrode). The cumulative volume of acid titrated was recorded at 5, 10, 15, 30, 45, 60, 75, 90, 105, and 120 min to provide a measure of the alkalinizing tendency (buffer release) by the shell powder. The equilibrating gas mixture flowed through the chamber at a measured rate throughout the titration period.

Samples of the incubating solution were taken before addition of shell and after 120 min of incubation and analyzed for the concentrations of Ca^{2+}, Mg^{2+}, Na^+, K^+, P_i, and lactate.

Shell Total CO_2 Determination. To determine total CO_2 of shell, a known mass (ca. 1 g) of dried powdered shell was incubated in 15 mL of 4 N HCl in the same flask as described above, and the CO_2 extracted by the ventilating gas was measured with a CO_2 analyzer (Applied Electrochemistry, model CD-3A) connected to a Kipp and Zonen recorder (model BD 41). The CO_2-evolution curve was integrated using a planimeter. Tests were made on all of the *Apalone* shells and on dried shell powder from five *Chrysemys picta bellii*. Water content of the *Chrysemys* shells were determined by oven drying.

Analytical Procedures

Processing of Bone and Shell Samples. Bone samples from both species and shell samples from *Chrysemys* were ground to powder at liquid N_2 temperature using a freezer mill (SPEX Certiprep, SPEX 6700-117). Weighed samples of powder were mixed with five parts of 8% perchloric acid and incubated at room temperature for 24 h before lactate analysis of the supernatant solution. Parallel samples of bone and shell were dried to constant weight to determine water content.

Softshell turtle shells, because of their higher water content, were dried before being ground to powder, and because of the nonhomogeneous nature of this shell, the entire shell of each animal was pulverized with the freezer mill and the resulting powder was thoroughly mixed before analysis. Samples of the homogenized powder were incubated with 12 parts 8% perchloric acid, and the supernatant solution was tested for lactate concentration. In an earlier study of *Chrysemys* shell (Jackson 1997), we found that predrying the shell did not affect the lactate analysis results. Additional weighed samples of shell powder were ashed in a muffle furnace at 450°C for 24 h to determine fractional weight of organic matter and ash in the shell, and a portion of the ash was dissolved in 12 parts 2 N HCl and analyzed for Na^+, K^+, total Ca^{2+}, total Mg^{2+}, and phosphorus, following suitable dilution.

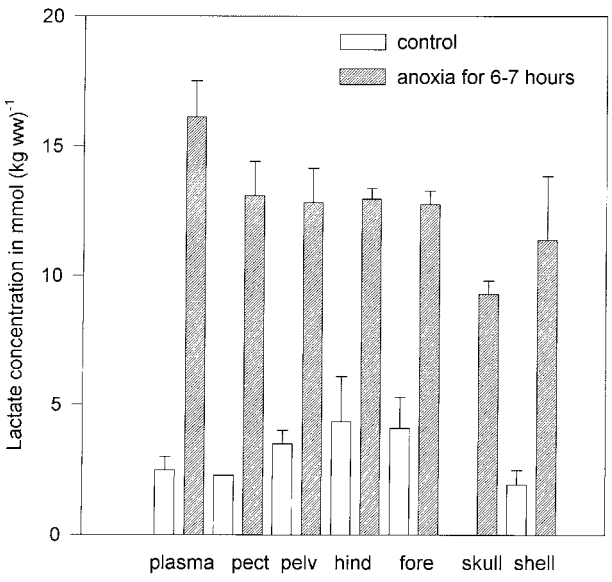

Figure 1. Lactate concentrations of plasma, shell, and selected bones from western painted turtles (*Chrysemys picta bellii*) under control conditions and after 6 h of submergence at 20°C. Bones analyzed were pectoral girdle (*pect*), pelvic girdle (*pelv*), hindlimb long bones (*hind*), forelimb long bones (*fore*), and skull. Note that no control sample was obtained for skull.

Table 1: Body mass, blood hematocrit, and plasma ion concentrations of the softshell turtle *Apalone spinifera aspera* and the western painted turtle *Chrysemys picta bellii* while normoxic and following submergence anoxia at 10°C for 2 d in *Apalone* and for 3, 6, and 9 d in *Chrysemys*

	Body Mass (g)	Hct (%)	$[Na^+]$	$[K^+]$	$[Ca^{2+}]$	$[Mg^{2+}]$	$[Lact^-]$
Apalone:							
Control ($N=5$)	202.2 ± 37.5	24.0 ± 1.6	105.7 ± 1.9	2.6 ± .2	1.7 ± .1	1.3 ± .04	1.8 ± .3
Anoxic (2 d; $N=4$)	248.4 ± 47.4	25.5 ± 3.4	106.9 ± 1.8	3.4 ± .5	3.5* ± .3	2.0* ± .1	35.6* ± 2.5
Chrysemys:							
Control 1 ($N=3$)	360.3 ± 18.6	15.0 ± 1.0	109.2 ± 1.6	1.6 ± .3	2.5 ± .1	2.2 ± .06	.9 ± .3
Control 2 ($N=4$)	490.3 ± 10.8	26.8 ± 3.5	106.7 ± 2.8	1.8 ± .1	2.4 ± .3	2.6 ± .1	.9 ± .2
Anoxic (3 d; $N=4$)	...	26.8 ± 4.5	104.1 ± 2.4	2.9 ± .4	4.7 ± .9	4.4 ± .3	30.8 ± 3.0
Anoxic (6 d; $N=4$)	...	21.8 ± 3.2	104.2 ± 3.4	3.4 ± .2	10.6 ± 1.3	7.4 ± .4	64.0 ± 6.2
Anoxic (9 d; $N=4$)	...	17.0 ± 4.2	97.0 ± 2.7	4.2* ± .3	18.1* ± 2.1	9.7* ± .6	83.4* ± 4.9

Note. Control 1 *Chrysemys* were killed in this state and tested for bone and shell lactate. Control 2 turtles were tested after 9 d of anoxia. Values are means ± SEM. Concentrations are in mmol L^{-1}.

* $P < 0.05$ compared to control.

Shell, Plasma, and Incubation Fluid Analysis. Plasma and shell lactates were analyzed either spectrophotometrically using Sigma reagents (test kit 826, Sigma Chemical, St. Louis) or with an automated lactate analyzer (YSI Stat Plus Analyzer, model 2300). Direct comparison of these methods on the same samples gave the same results. Sodium and potassium were measured using a flame photometer (IL model 943), calcium and magnesium using an atomic absorption spectrophotometer (Perkin-Elmer, model 280), and inorganic phosphorus using the Fiske and SubbaRow method with Sigma reagents and a spectrophotometer.

Statistics

Results are presented as mean ± SEM. Differences between control and anoxic values for each species were evaluated using *t*-tests, and comparisons of results from the two species were also compared using *t*-tests. Possible differences in distribution of lactate throughout the skeleton of *Chrysemys* were tested by ANOVA. All tests were performed using SigmaStat software (Jandel Scientific, San Rafael, Calif.).

Results

Distribution of Lactate in Chrysemys *Bone and Shell*

Following 6 h of apneic submergence of *Chrysemys* at 20°C, lactate distributed throughout the shell and bone in a nearly uniform manner (Fig. 1). Anoxic shell and bone values were significantly higher than normoxic values from the same site. Anoxic bone and shell values (mmol kg^{-1}), however, were not significantly different from plasma values (mmol L^{-1}). Because lactate entered all parts of the skeleton, we assumed that the same was true in this species at 10°C and in *Apalone* at 10°C and, therefore, analyzed hindlimb bone as a representative skeletal sample. We cannot be sure, however, that the distribution was uniform in these other situations.

Blood and Plasma Values of Anoxic Turtles at 10°C

The pattern of changes observed in plasma ions (Table 1) were similar to previous observations on these species under similar conditions (Jackson et al. 1984). In *Chrysemys*, significant increases were observed in the concentrations of lactate, K^+, total Ca^{2+}, and total Mg^{2+} ($P < 0.01$ for all ions). The apparent fall in $[Na^+]$ was not significant. The observed increases in K^+, Ca^{2+}, and Mg^{2+} can be regarded as compensatory to the lactate increase, serving to preserve plasma strong ion difference (Stewart 1983). In *Apalone*, the changes were more modest, although the increases in the concentrations of lactate, total Ca^{2+}, and total Mg^{2+} were significant (*t*-test, $P < 0.01$). Neither $[K^+]$ nor $[Na^+]$ changed significantly.

Bone and Shell Lactate in Chrysemys *and* Apalone *at 10°C.* Lactate levels increased significantly in plasma, shell, and hindlimb bone in both *Chrysemys* and *Apalone* submerged in anoxic water at 10°C (Figs. 2 and 3). In *Chrysemys*, bone lactate was significantly higher than shell lactate ($P = 0.003$), but neither bone nor shell were significantly different from plasma. In *Apalone*, both bone and shell and were significantly lower than plasma ($P < 0.05$) but were not different from each other. Lactate levels in *Chrysemys* were significantly higher than in *Apalone* associated with the longer duration of anoxia. Mean rates at which lactate concentration increased, calculated as mmol kg h^{-1}, however, were lower in *Chrysemys*. Rates for plasma, shell, and bone, respectively, were 0.38, 0.31, and 0.41 in *Chrysemys* and 0.72, 0.41, and 0.51 in *Apalone*.

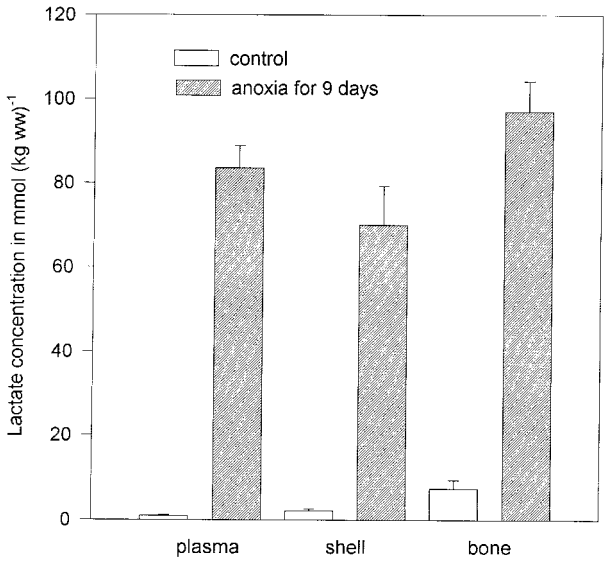

Figure 2. Lactate concentrations of plasma, shell, and hindlimb bones of western painted turtles (*Chrysemys picta bellii*) at 10°C while normoxic and after 9 d of anoxic submergence.

Shell Composition in Chrysemys *and* Apalone

Shell characteristics were measured on all specimens of *Apalone* and tabulated values compare normoxic and anoxic groups (Table 2). Thorough shell analysis was not performed on the specimens of *Chrysemys* from this study, so values presented in Table 2 are control values from turtles acclimated at 3°C from Jackson et al. (2000). The relatively small fraction of *Apalone* shell that is mineralized was revealed by the smaller ash percentage and the larger water percentage. Also, shell concentrations of the elements, expressed per gram wet weight, were significantly lower in *Apalone* than in *Chrysemys* (with the exception of K^+). Calcium and phosphorus, the major constituents of shell in both species, were only 20%–25% as plentiful in *Apalone* shells, although the abundance, in millimoles per kilogram ash, was similar in the two species (calcium: 8,800 ± 700 in *Apalone* vs. 9,400 ± 300 in *Chrysemys*). This suggests that the mineralized portions of the shells of the two species are similar, but that *Apalone* has less of its shell in this form. The shells of both turtles accounted for about the same percentage of the respective body masses.

In Vitro Shell Incubation

Powdered shell from *Apalone* alkalinized the incubating solution, but the rate of HCl titration required to maintain constant solution pH was significantly less ($P < 0.02$) at both pH 6.0 and 6.5 than previously observed with shell from *Chrysemys* (Jackson et al. 1999), even expressed on a dry weight basis (Fig. 4*A*). The difference in the two species was even more dramatic when compared on the basis of calculated wet weight. In this case, *Apalone* shell required only about 25% as much HCl titration (Fig. 4*B*). By the end of the 2-h incubation period, solution $[Ca^{2+}]$ and $[Mg^{2+}]$ had both increased: at pH 6.0 to 24.0 ± 2.0 and 4.9 ± 0.4 mmol L^{-1}, respectively, and at pH 6.5 to 14.8 ± 0.9 and 4.1 ± 0.2 mmol L^{-1}, respectively. Only a negligible increase was observed in solution inorganic phosphate concentration. For the five normoxic shell incubations at pH 6.0, mean $[P_i]$ after 2 h was 0.038 mmol. Solution lactate concentration fell by 4.8 ± 0.8 mmol L^{-1}, consistent with lactate uptake by shell during incubation. This is significantly less uptake than we previously observed in *Chrysemys* shell powder at pH 6.5, in which solution lactate concentration fell by 14.6 ± 0.8 mmol L^{-1}.

Shell total CO_2 concentration from the five normoxic *Apalone* was 0.754 ± 0.036 mmol g dw^{-1} (dw = dry weight), significantly less ($P < 001$) than in shell samples from five normoxic *Chrysemys* (1.259 ± 0.011 mmol g dw^{-1}). When corrected to fresh wet weight on the basis of initial water content, *Apalone* shell was calculated to contain only 20% as much CO_2 per gram as *Chrysemys*.

Discussion

As previously observed (Jackson et al. 1984; Ultsch et al. 1984) and confirmed in this study, when *Apalone* and *Chrysemys* are

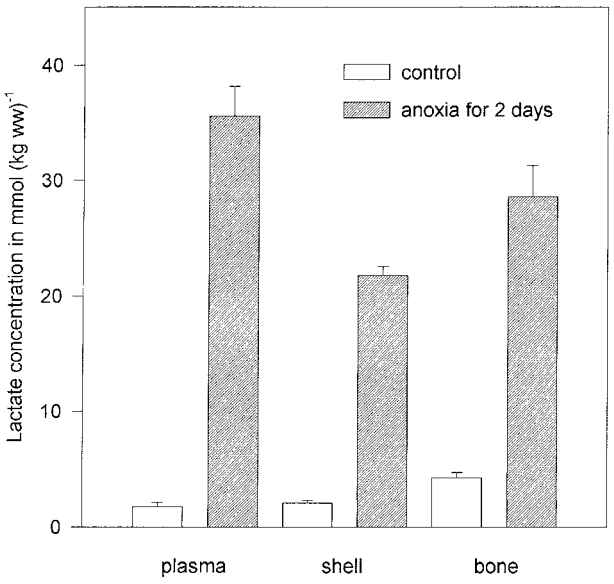

Figure 3. Lactate concentrations of plasma, shell, and hindlimb bones of spiny softshell turtles (*Apalone spinifera aspera*) at 10°C while normoxic and after 2 d of anoxic submergence.

Table 2: Composition of shells from the softshell turtle *Apalone spinifera aspera* and the western painted turtle *Chrysemys picta bellii*

	Body Mass (g)	Shell Mass (% BM)	Shell Water (%)	Shell Ash (%)	Shell $[Na^+]$	Shell $[K^+]$	Shell $[Ca^{2+}]$	Shell $[Mg^{2+}]$	Shell $[P_i]$
Apalone:									
Normoxic									
($N = 5$)	202.2 ± 37.5	31.4 ± .6	76.9 ± 3.1	9.0 ± 1.5	77.1 ± 8.0	8.0 ± 1.0	790 ± 130	19 ± 3	401 ± 80
Anoxic									
($N = 4$)	284.4 ± 47.4	29.6 ± 1.4	74.1 ± 2.8	10.9 ± 1.7	77.9 ± 5.6	7.8 ± .4	1,040 ± 160	21 ± 1	506 ± 71
Chrysemys:[a]									
Normoxic									
($N = 5$)	766.2 ± 68.9	32.4 ± 1.0	27.9 ± .6	44.7 ± .8	162.1 ± 4.9	4.8 ± .6	4,200 ± 80	102 ± 1	2,100 ± 30

Note. Values are means ± SEM. Concentrations are in mmol kg shell ww^{-1}.

[a] From study by Jackson et al. (2000) on 3°C turtles.

each submerged in anoxic water at 10°C, plasma lactate concentration rises at a faster rate in *Apalone* and plasma $[Ca^{2+}]$ and $[Mg^{2+}]$ rise at a slower rate. Together, these differences in performance contribute to a more rapid development of metabolic acidosis in *Apalone* and shorten the time this turtle can withstand anoxic stress. The rate of increase in plasma lactate concentration we observed here was about twice as fast in *Apalone* as in *Chrysemys*; in our earlier study (Jackson et al. 1984), this difference was even greater, more than threefold higher in *Apalone*. We interpret these differences to indicate a significantly higher rate of anaerobic metabolism in submerged *Apalone*. The magnitude of the ionic compensatory response to lactic acidosis was also less in *Apalone*. Although both plasma Ca^{2+} and Mg^{2+} levels increased during anoxia, these changes were not as great as observed in *Chrysemys*, even at the same approximate increase in plasma lactate concentration. The net total increase in $[Ca^{2+}]$ plus $[Mg^{2+}]$ averaged 2.5 mmol L^{-1} in *Apalone* with a plasma lactate concentration of 35.6 mmol L^{-1}, whereas these ions rose by a total of 4.0 mmol L^{-1} in *Chrysemys* at a lactate level of only 30.8 mmol L^{-1}. In an earlier study (Jackson et al. 1984), the increase in the two ions at a common plasma lactate of 26.7 mmol L^{-1} was 5.8 mmol L^{-1} for *Chrysemys* but only 1.6 mmol L^{-1} for *Apalone*.

The smaller compensatory release of calcium and magnesium into the blood by *Apalone* is consistent with the substantially smaller degree of mineralization of this turtle's shell. As described by Zangerl (1969), the Trionchidae lack both the peripheral dermal ossification and the overlying calcified epidermal plates of most turtles. Mineralized components in the shell are largely skeletal elements fused into a structure composed primarily of connective tissue with overlying skin. Our measurements reveal that the shell of *Apalone*, though nearly the same fraction of body mass as in *Chrysemys*, has only 20%–25% as much calcium and phosphorus content, consistent with reduced mineralization. Assuming that the remaining skeleton of *Apalone* (outside the shell) has a fresh mass of about 5.5% of body mass as in *Chrysemys* (Jackson 1997), then total bone mass of *Apalone* would be close to 12%, not far from the predicted skeletal size for a similarly sized snake or mammal (Calder 1984). This is far less than the bone mass (shell + skeleton) of *Chrysemys*, which is 35%–40% of body mass (Jackson 1997).

The larger bone mass in *Chrysemys* appears to afford this turtle a clear advantage in supplementing its extracellular buffering capacity with calcium and magnesium carbonates. This is borne out by pH-stat incubations of shell powder from the two species. *Apalone* shell released only 25% as much base per gram wet weight as we had previously observed on *Chrysemys* shell (Jackson et al. 1999). This conforms closely to the lower shell content of bone mineral noted above. Furthermore, as in *Chrysemys* shell (Jackson et al. 1999), the base is released from *Apalone* shell primarily in the form of carbonates. We observed only a slight increase in solution $[P_i]$, indicating that the apatite crystal was not significantly broken down by the acid environment. In *Chrysemys*, plasma $[P_i]$ did not change during five mo of anoxic submergence at 3°C (Jackson et al. 2000), indicating that shell and bone calcium phosphate remains unaffected by acidosis in vivo, as well. We conclude, therefore, that the shells of both turtles release calcium and magnesium carbonates in response to an acid environment, but the release from *Apalone* is less because of the lower mineral content in this animal's shell.

The shells of both species accumulated lactate during anoxia, despite their very different compositions. In *Chrysemys*, shell lactate reached levels (mmol g ww^{-1}; ww = wet weight) approximating plasma concentrations (in mmol L^{-1}), despite a lower water content (~30%). As discussed in an earlier paper (Jackson 1997), it is probable that shell lactate in this turtle exists in combined form, perhaps complexed with Ca^{2+}, within the shell, rather than in simple solution. This may not be the case with *Apalone*, however, except for the fraction of shell lactate within the bone proper. Most of shell lactate in this species may be dissolved in the shell water, which comprises over 75% of fresh shell mass. This interpretation is supported

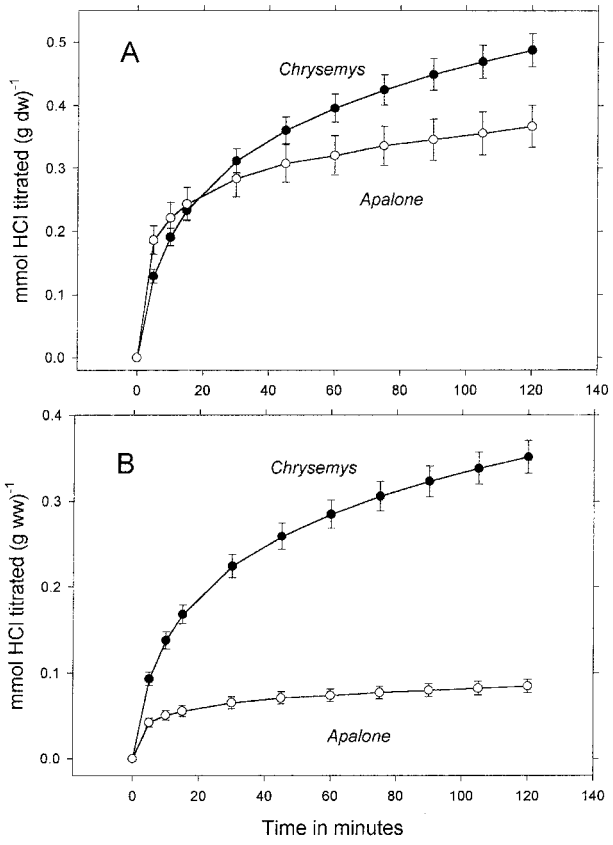

Figure 4. Cumulative titration volume of 1 N HCl required to maintain solution pH at 6.5 during incubation of shell powder from spiny softshell turtles (*Apalone spinifera aspera*) and western painted turtles (*Chrysemys picta bellii*). *Chrysemys* data is from Jackson et al. (1999). Panel *A* depicts results expressed per gram dry shell, and panel *B* depicts results expressed per gram wet shell.

by the smaller lactate uptake by dry shell powder compared to *Chrysemys*, because the uptake in this case may be limited by the available calcium for lactate binding. The relatively low ratio of shell K^+ to shell Na^+ content in *Apalone* (Table 1) suggests that most shell water is extracellular, but the low shell lactate concentration relative to plasma concentration in *Apalone* may also indicate a low perfusion of this structure during anoxic submergence. The uptake of lactate by *Apalone* shell powder in vitro was significantly less than uptake previously observed in *Chrysemys*.

This study also establishes that the skeleton outside the shell accumulates lactate during submergence anoxia. We have also recently documented this in *Chrysemys* during anoxic submergence at 3°C (Jackson et al. 2000). Given the similar composition of skeletal bone and calcified shell, this is not an unexpected finding, but these are the first studies to our knowledge, in which this potentially important phenomenon has been documented. The data from anoxic *Chrysemys* at 20°C also reveal that the entire skeleton participates in this regard, and the hindlimb measurements at 10°C indicate that bone lactate uptake occurs comparably in *Chrysemys* at this temperature and in *Apalone*. In both species, moreover, the bone uptake appeared to exceed shell uptake, although this was only significant in *Chrysemys*. The basis for a preferential uptake by skeleton is not clear, although it is possible that perfusion of bone, and therefore exchange with blood, is more effective than in shell.

The contribution of bone to lactic acid sequestration and buffering extends the importance of this mechanism in turtles such as *Chrysemys*. In this species, the shell is about 32% of body mass and the balance of the skeleton is another 5.5% (Jackson 1997). The combined mass provides not only a huge reservoir of potential buffering power for export to the extracellular fluid, but also a large site for the deposition and buffering of lactic acid. Using estimates of intracellular and extracellular fluid volumes presented earlier (Jackson 1997), we calculate that some 44% of the total body lactate of *Chrysemys* after 9 d anoxia at 10°C resided in shell and bone, and in vitro measurements indicate that the associated protons are buffered there (Jackson et al. 1999). Furthermore, as discussed earlier (Jackson 1997), buffer release into the extracellular fluid from shell and bone neutralizes another 20% or so of the total body lactic acid so that the total contribution of the shell and bone to lactic acid buffering is close to two-thirds of the total.

Because estimates of extracellular and intracellular fluid volumes are not available for *Apalone*, similar calculations for this species cannot be made with any confidence, but the lower buffer capacity and the somewhat smaller relative uptake of lactate by shell and bone demonstrate that the shell of this turtle makes a much smaller contribution to overall lactic acid buffering. Consequently, preexisting buffers of the intra- and extracellular fluids of *Apalone* must neutralize a greater fraction of the generated lactic acid and, consequently, are liable to be depleted more rapidly. When the faster rate of lactic acid production is also brought into the picture, it becomes clear why the tolerance of *Apalone* to anoxia is inferior to *Chrysemys*.

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