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THE EFFECTS OF LOW pH ON LACTATE DEHYDROGENASE KINETICS OF DIVING AND NONDIVING REPTILES

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ABSTRACT

The properties of lactate dehydrogenase were examined in two snake species, *Nerodia rhombifera* and *Elaphe obsoleta*, and a turtle species, *Pseudemys scripta*. Our purpose was to compare the LDH activity of reptiles with limited anaerobic capabilities with that of the well established diver *Pseudemys*. The Michaelis-Menten kinetics of LDH and its susceptibility to inhibition by elevated pyruvate concentrations were investigated in the brain and heart of the three species. All tissue incubations and enzyme activity determinations were done at a pH of 7.0 in order to stimulate a diving blood pH in the three species.

In both tissues the LDH activity of the snakes was higher than that of *Pseudemys* at pyruvate concentrations ranging between .03 mM and .50 mM. The K_m values of the snakes were lower than those of *Pseudemys*, suggesting a greater enzyme-substrate affinity in the snake tissues. The V_{max} values were higher in the snake tissues indicating a faster conversion of substrate to product.

Heart LDH activity was reduced to an equal extent by high pyruvate concentrations in each of the three species. *Elaphe* brain LDH was most susceptible to pyruvate inhibition, but *Nerodia* and *Pseudemys* brain LDH were inhibited to an equal extent.

The results indicate that the kinetic behavior of brain and heart LDH of the three species is similar at a pH of 7.4 and a pH of 7.0. The results also suggest that the LDH of *Pseudemys* is no better adapted to withstand anaerobic conditions than that of *Nerodia* or *Elaphe* at a pH of 7.0.

INTRODUCTION

Representative species of each of the vertebrate classes have the ability to remain submerged for extended periods of time. These animals are commonly referred to as the diving animals. In the past the diving birds and mammals have received the most attention, but more recently many studies have focused on diving reptiles.

The diving capabilities of sea snakes are well documented (Graham, 1974; Heatwole and Seymour, 1975; Heatwole, 1975). Less aquatic snakes can also remain submerged for periods in excess of 1 hour (Baeyens et al., 1980). The turtles are, however, the best reptilian divers. Many fresh water turtles can tolerate periods of submergence of several hours at summer temperatures (Burggren and Shelton, 1979; Lucey and House, 1977; Penney, 1974) while at winter temperatures they can remain submerged for periods of four to six months (Musacchia, 1959; Ultsch and Jackson, 1982).

The diving capabilities of reptiles have been attributed to various physiological adaptations, but the most important factor prolonging dive times in reptiles is an especially pronounced ability to liberate energy through anaerobic metabolism (Jackson, 1968). The tissue enzymes of diving reptiles appear to have special properties that enable them to liberate large quantities of ATP by anaerobic means (Lutz et al., 1978; Simon et al., 1979; Storey and Hochachka, 1974).

The kinetic properties and susceptibility to substrate inhibition of the glycolytic enzyme, lactate dehydrogenase (LDH), have been studied in heart and brain of *Nerodia rhombifera*, *E. obsoleta* and *Pseudemys scripta* (Baeyens et al., 1985). These studies were carried out at a pH of 7.4 which simulates a nondiving blood pH in these three species. The results indicated that the kinetic behavior of the enzyme was similar in the three species.

The present study examines LDH in heart and brain of *N. rhombifera*, *E. obsoleta* and *P. scripta*. All incubations and enzyme analyses were done at a pH of 7.0, which simulates a diving pH in the three species. We examined the Michaelis-Menten kinetics of LDH and the susceptibility of LDH to pyruvate inhibition. It was hoped that examining the properties of tissue LDH would lead to a better understanding of the difference in anaerobic threshold between the snakes and *Pseudemys*.

MATERIALS AND METHODS

N. rhombifera (55-63 cm snout vent length [SVL]) were collected from minnow ponds in Lonoke County, Arkansas. *E. obsoleta* (80-105 cm SVL) were collected from wooded areas in Pulaski County, Arkansas. *P. scripta* were obtained from commercial dealers. All animals were maintained at room temperature (20-25 °C) and were allowed at least 30 days to acclimate to captive conditions before they were used for experimentation.

Enzyme activity was measured in brain and heart homogenates of the three species. Four individuals of each species were used for enzyme analysis. After determining that there was no significant variation in LDH activity for a specific tissue within a species, the results obtained for the four individuals of that species were pooled and compared with the results obtained from the other two species. Enzyme activity was expressed as a change in absorbency/min/mg protein. Protein concentration was determined by the method of Lowry et al. (1951) using bovine serum albumin as a standard. The differences between the means of K_m and V_{max} values were analyzed by Student's t-tests.

Tissue preparation

Animals were killed by cervical dislocation and samples of brain and heart were immediately removed from the animal. The tissue samples were weighed to the nearest mg and then homogenized in 0.1 M phosphate buffer (pH 7.0) in a ratio of 1 g of tissue to 7 ml of buffer. After homogenization the samples were centrifuged at 2500 x g for 40 minutes. The supernatant was stored at -80 °C. Preceding enzyme analysis the supernatant was further diluted with 0.1 M phosphate buffer in a ratio of 1 part homogenate to 9 parts phosphate buffer.

LDH activity determinations

LDH activity was measured by spectrophotometrically recording the oxidation of NADH to NAD⁺ with a Varian dual beam spectrophotometer (model DMS90). The assay mixture consisted of 2.8 ml of 0.1 M phosphate buffer (pH 7.0), 100 μ l of sodium pyruvate of variable concentration, and 100 μ l of 6.6 mM NADH. The reaction was initiated by adding 10 μ l of the tissue homogenate to the mixture in a cuvette. After mixing, the decrease in absorbency was measured over a 5 minute period.

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Six concentrations of pyruvate, ranging from 0.03 mM to 0.5 mM, were used to examine the Michaelis-Menten kinetics of LDH. Lineweaver-Burk plots were constructed from the means of four measurements at each pyruvate concentration. Apparent K_m and V_{max} values were calculated from the plots.

Susceptibility of LDH to substrate inhibition was determined by varying the concentration of pyruvate in the reaction mixture between 0.16 mM and 6.6 mM. Four measurements were made at each of six different substrate concentrations within this range and the results were averaged for each concentration.

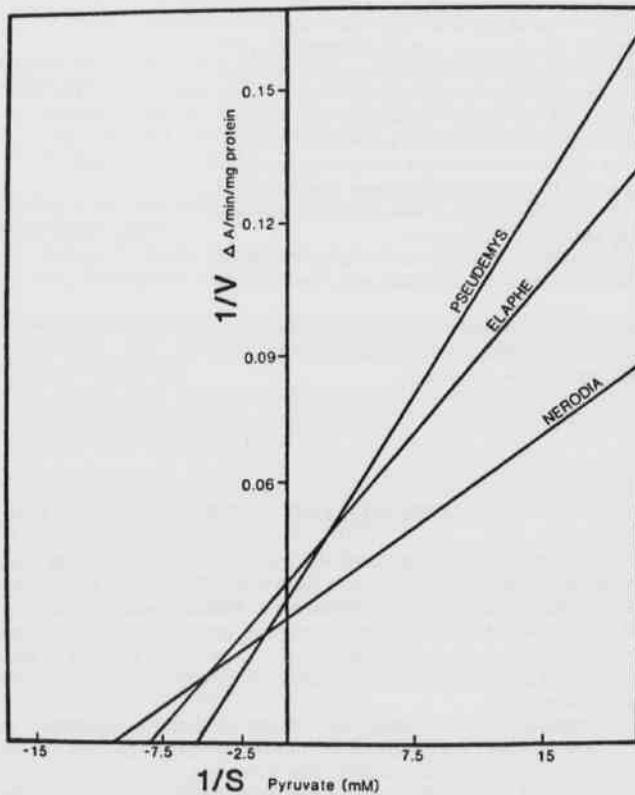


Figure 1. Lineweaver-Burk plot of brain LDH activity in *N. rhombifera*, *E. obsoleta* and *P. scripta*.

Table 1. Summary of the brain and heart LDH kinetic data in *N. rhombifera*, *E. obsoleta* and *P. scripta* at pH 7.0.

SPECIES	TISSUE	V_{max}	K_m
<i>N. rhombifera</i>	Brain	27.03	2.08**
<i>E. obsoleta</i>	Brain	21.28*	2.68
<i>P. scripta</i>	Brain	23.26	4.00
<i>N. rhombifera</i>	Heart	71.43	5.56
<i>E. obsoleta</i>	Heart	71.43	7.69
<i>P. scripta</i>	Heart	62.50***	7.69***

*Significantly different from *P. scripta* and *N. rhombifera* brain ($P < 0.05$)

**Significantly different from *P. scripta* brain ($P < 0.05$)

***Significantly different from *N. rhombifera* and *E. obsoleta* heart ($P < 0.05$)

****Significantly different from *N. rhombifera* heart ($P < 0.05$)

RESULTS

Kinetic Studies

Figures 1 and 2 represent Lineweaver-Burk plots for brain and heart LDH of the three species. *Nerodia* brain LDH activity was highest throughout the range of pyruvate concentrations utilized, while *Pseudemys* brain had the lowest activity (Fig. 1). The V_{max} value of *Nerodia* brain was also the highest of the three species. In contrast, the apparent K_m value of *Nerodia* was the lowest of the three species while that of *Pseudemys* was the highest.

The kinetics of heart LDH are shown in Figure 2. *Nerodia* had the highest activity throughout the range of substrate concentrations and *Pseudemys* had the lowest. The V_{max} values were greater in the two snakes than in *Pseudemys*. The apparent K_m of *Nerodia* was lower than that of *Pseudemys* and *Elaphe*. The K_m and V_{max} values for brain and heart are summarized in Table 1.

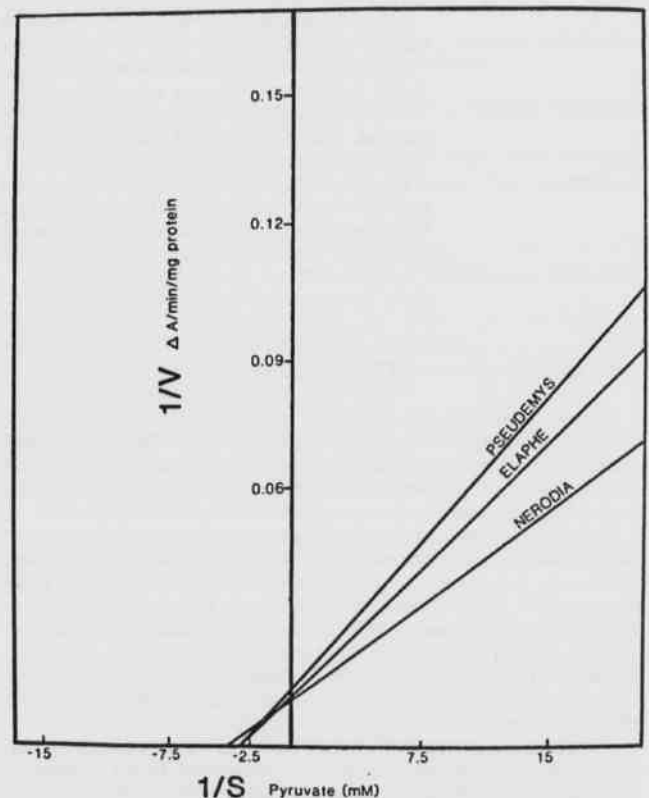


Figure 2. Lineweaver-Burk plot of heart LDH activity in *N. rhombifera*, *E. obsoleta* and *P. scripta*.

Substrate Inhibition Studies

Highest brain LDH activity occurred at a 0.5 mM pyruvate concentration in all three species (Fig. 3). At higher substrate concentrations there was a progressive and equal reduction in enzyme activity in *Nerodia* and *Pseudemys*. The degree of substrate inhibition was greater in *Elaphe* than in either of the other two species.

Highest heart LDH activity occurred at a 1.0 mM pyruvate concentration in *Pseudemys*, a 1.33 mM pyruvate concentration in *Elaphe* and 1.66 mM pyruvate concentration in *Nerodia* (Fig. 4). At substrate concentrations between 1.0 mM and 3.33 mM *Pseudemys* demonstrated the greatest degree of substrate inhibition. At substrate concentrations in excess of 3.33 mM the degree of substrate inhibition was approximately equal in the three species.

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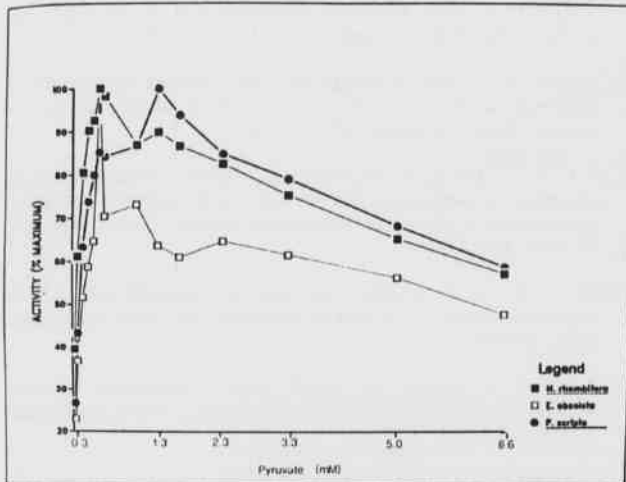


Figure 3. Substrate inhibition plot of brain LDH activity in *N. rhombifera*, *E. obsoleta* and *P. scripta*.

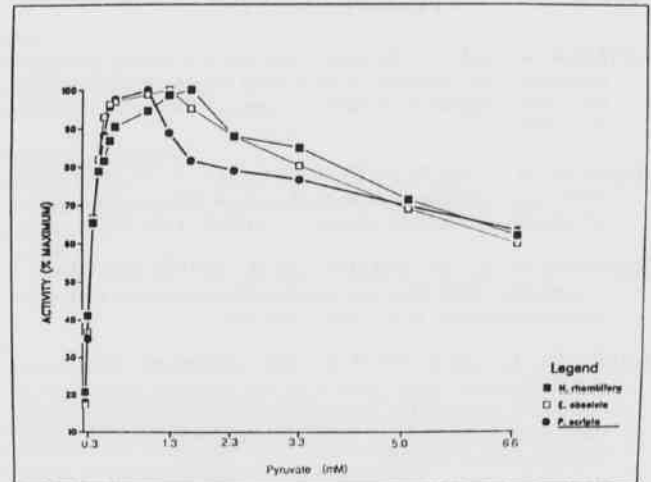


Figure 4. Substrate inhibition plot of heart LDH activity in *N. rhombifera*, *E. obsoleta* and *P. scripta*.

DISCUSSION

An increased reliance on anaerobic glycolysis during diving seems to be of particular importance in extending the dive times of reptiles. Belkin (1962) found that blocking glycolysis with iodoacetate in the loggerhead musk turtle, *Stenothaerus minor*, resulted in a significant decrease in underwater survival time. Similarly, blocking glycolysis in the river cooter, *Pseudemys concinna*, permitted indefinite survival in air but rendered the animal incapable of prolonged diving (Belkin, 1961).

An increased reliance on anaerobic glycolysis during diving is also indicated by the increased levels of pyruvate and lactate which accumulate in the blood and tissues. Altman and Robin (1969) noted progressive increases in blood pyruvate and lactate while diving for a 24 hour period in *P. scripta*. There was an approximate doubling of the pyruvate level and the lactate level increased over 8-fold. Clark and Miller (1973) found increases in the levels of pyruvate and lactate in brain, heart and liver of *P. scripta* following a 3 hour period of anaerobiosis. The pyruvate levels increased in each tissue, more than doubling in brain and heart to values in excess of $0.2 \mu\text{M/g}$, while the lactate levels increased from approximately $5 \mu\text{M/g}$ to over $40 \mu\text{M/g}$ in the same time period. Similar increases in blood lactate and pyruvate during diving have been reported in the sea turtle, *Chelonia mydas*, (Berkson, 1966; Hochachka et al., 1973) and the western painted turtle, *Chrysemys picta* (Jackson and Heisler, 1982). Diving induced increases in blood lactate have also been observed in snakes. Seymour and Webster (1975) measured blood lactate in four species of sea snakes (following forced dives of 0.5 hour duration and found that the values increased from pre-dive levels of 10 mg% to over 60 mg%. We have found similar increases in blood lactate in *N. rhombifera* following 20 minute forced dives in our laboratory (unpublished observation).

In addition to the accumulation of pyruvate and lactate, there is a concomitant decrease in blood pH as a result of diving. Berkson (1966) found that the arterial pH of the Pacific green turtle, *Chelonia mydas*, fell from a pre-dive value of 7.4 to approximately 7.0 after 1 hour of submergence. Likewise, Utsch et al. (1984) measured precipitous drops in blood pH, to values approaching 7.0, in four species of fresh water turtles during period of anaerobiosis.

The increased accumulation of pyruvate and lactate with the fall in blood pH suggests a greater glycolytic activity during diving. Miller and Hale (1968) measured the activity of the glycolytic enzyme LDH in *P. scripta* and rat brain. They found significantly greater enzyme activity in *Pseudemys* brain and concluded that this was at least partially responsible for the ability of the turtle brain to withstand longer periods of anaerobiosis.

The Michaelis-Menten kinetics of LDH in brain and heart of *N. rhombifera*, *E. obsoleta* and *P. scripta* have been compared at a pH of 7.4 in a previous study (Baeyens et al., 1985). The results of that study indicated that there was no relationship between the LDH activity of brain and heart and the ability to withstand anaerobic conditions in the three species.

In the present study, carried out a pH of 7.0, we found that there was less LDH activity in brain and heart of *Pseudemys* than in either *Nerodia* or *Elaphe* at pyruvate concentrations ranging between 0.03 and 0.50 mM. Furthermore, the K_m values of snake brain and heart were lower than those of *Pseudemys* suggesting a greater affinity of LDH for pyruvate in the snakes. Finally, the higher V_{max} values of snake brain and heart suggest a faster conversion of pyruvate to lactate when the enzyme is saturated with substrate. Thus, the enzyme demonstrates similar kinetic behavior at a pH of 7.4 and a pH of 7.0 in brain and heart and there appears to be no correlation between LDH activity and anaerobic threshold in the three species.

The capability of prolonged underwater survival may be related to the tissue distribution of LDH isozymes in diving reptiles. For example, Miller and Hale (1968) found a higher proportion of M subunits in brain and heart of *P. scripta* than in the same tissues of the albino rat. Furthermore, the proportions of M subunits in turtle brain, heart and skeletal muscle were similar to those in mammalian skeletal muscle. Since mammalian skeletal muscle is highly adapted to anaerobic conditions, they reasoned that the turtle tissues were similarly adapted.

Altman and Robin (1969) found that both heart and skeletal muscle LDH of *P. scripta* had similar proportions of H and M subunits. In addition, the pyruvate inhibition patterns were virtually identical in both tissues. In a related study, the properties of LDH M and H subunits were examined in liver and skeletal muscle of the marine turtle *Caretta caretta* (Baldwin and Gyuris, 1983). Using purified H_4 and M_4 isozymes they found that the H_4 activity progressively fell with increasing pyruvate concentrations. In contrast, the M_4 activity was completely insensitive to substrate concentration.

Having LDH with a high M subunit activity would clearly be advantageous during a dive because of its ability to remain functional in the presence of high substrate concentrations. Comparisons of heart LDH activity in the present study reveals that *Pseudemys* LDH is no more resistant to substrate inhibition than is the snake enzyme. Likewise, in brain the degree of substrate inhibition was no greater in *Nerodia* than in *Pseudemys*. These findings suggest that a possible M subunit adaptation to anaerobic conditions may be important in extending the dive times in all three species. The results also indicate that the greater dive times of *Pseudemys* cannot be explained solely on the basis of a unique M subunit adaptation in this species.

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