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ANAEROBIC METABOLISM AND MAXIMAL RUNNING IN THE SCORPION *CENTRUROIDES HENTZI* (BANKS) (SCORPIONES, BUTHIDAE)

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ABSTRACT. When forced by prodding to run continuously, *Centruroides hentzi* (Banks 1901) (Scorpiones, Buthidae) lost over 70% of initial speed within 30 s and moved fitfully, if at all, after 90 s. A lack of behavioral response to alternative stimuli presented after two mins of prodding suggested that the scorpions were physiologically fatigued. Mean whole body D-(-)-lactate concentration increased from resting values of 0.6 μmol/g to 4 μmol/g at exhaustion, an approximately 6.5-fold change. It is unlikely that scorpions accumulate significant amounts of other anaerobic products. Whole body lactate accumulations in *C. hentzi* are lower than those found in species of spiders, crabs and terrestrial ectothermic vertebrates that are more specialized for running. This difference may be the result of proportionately more non-locomotory body mass in the bodies of scorpions compared to these other animals and not due to lower rates of anaerobic metabolism within locomotory muscles.

Keywords: D-(-)-lactate, running, exercise

Scorpions have low resting rates of aerobic metabolism; typically only 25% of many other terrestrial arthropods (Anderson 1970; Lighton et al. 2001). This, combined with a low-activity sit-and-wait or slow search predatory style, means that total energy expenditures are small. The result is that scorpions are able to endure periods of low food availability and to convert a large portion of their food into biomass or progeny (Lighton et al. 2001). In some arid ecosystems scorpion biomass may exceed that of all vertebrates combined (Polis & Yamashita 1991).

For any animal to engage in vigorous activities such as prey capture or predator avoidance, it must be able to provide ATP at rates that match the demands of active muscles. Good, albeit limited, evidence from vertebrates suggests that animals with low resting rates of aerobic metabolism also have low maximum rates of aerobic metabolism (Rezende et al. 2004). Moreover, in burst activity there is little time to fully activate the subcellular and organ system components of aerobic metabolism. In most groups of animals, sudden onset, high power requirements are largely met by anaerobic glycolysis and the depletion of high-energy phosphate storage compounds such as arginine phosphate or creatine phosphate (McArdle et al. 2001). Within the arachnids, the importance of these pathways is well documented in spiders (Prestwich 1983a, b, 1988a, b). Scorpions possess respiratory and circulatory structures that are similar to those of spiders, and, like many spiders, they are not highly active predators. Given these similarities and their low resting rates of metabolism, it is reasonable to expect that scorpions would also rely on anaerobic metabolism during intense activity.

The only previous data on anaerobic metabolism in scorpions was the finding by Long & Kaplan (1968) that *Centruroides sculpturatus* Ewing (1928), possessed a high activity of the enzyme D-(-)-lactate dehydrogenase (dLDH). This same enzyme is found in high activity in spiders (Long & Kaplan 1968; Prestwich & Ing 1982) where it is associated with production of D-(-)-lactate at rates that depend on the species and intensity of activity; measurable accumulations may be found after 5 to 10 s of intense running or struggle (Prestwich 1983a,b, 1988a,b). The work presented in this paper confirms that at least one species of scorpion also produces substantial amounts of D-lactate during forced running.

METHODS

Animals.—*Centruroides hentzi* (Banks 1901), also referred to as Hentz's striped bark

scorpion, is a small scorpion whose geographic range is essentially restricted to Florida (Shelley & Sissom 1995). It has a low resting rate of metabolism (Anderson 1970). I collected adults from under the bark of dead trees in pasture parklands and woods located within 15 km of location 29.671° N, 82.458° W (northwest of Gainesville, Alachua County, Florida). Since all individuals were used destructively (see below), there are no voucher specimens. I housed the scorpions individually in plastic cages containing dry sand, pine bark, and a water source at 25 °C on a 12L: 12D schedule and fed them early instar crickets every four days. The last feeding was five days prior to their use in an experiment so as to put all in a comparable nutritional state (Anderson 1974).

Forced Running Performance.—I forced all individuals to run in a rectangular arena measuring 1.0 (L) × 0.3 (W) × 0.2 (H) meters that had an interior marked in 0.1 m grids. I stimulated running by touching their telsons with a blunt rod. I determined running speeds by measuring the distance run over 5 s and dividing by time to obtain speed in m/s. I divided this result by the body length. All exercise took place at 25 °C.

To be sure that what appeared to be fatigue was not merely habituation to prodding, I exercised five individuals by prodding them until they moved only very slowly and then brought a hot soldering iron near them (they were not touched). In rested individuals this heat stimulus always produced rapid running.

Anaerobic Metabolism.—The day after the running speed measurements, all individuals were used destructively to obtain lactate samples in one of four separate treatment groups: two rest groups and two exercise groups. There were no statistically significant differences in the masses of the individuals in each group (one-way ANOVA, $P = 0.28$, 16, 3 *df*). I observed the resting individuals for about one hour to be sure that they did not struggle or make more than minor, isolated movements. I then froze them by immersion in liquid N_2. I ran the exercise groups in the arena as described above for two mins and then also froze these individuals in liquid N_2.

I homogenized the frozen members of one rest and exercise group in ice-cold 10% tricholoroacetic acid (TCA) and the remaining rest and exercise groups in ice-cold 0.6 M $HClO_4$. The difference in procedure is necessitated by the two different methods of analysis I used for lactate; TCA was not consistent with the enzymatic techniques and $HClO_4$ was not consistent with the colorimetric technique. Protein precipitants from these acid treatments were removed by filtration (Prestwich 1983a).

I analyzed the deproteiniated filtrates of $HClO_4$-treated rest and exhaustion samples enzymatically for both D- and L-lactate using the method of Gawehn & Bergmeyer (1974). To do this, I performed the assay the same way except that in one case I used the D-optical isomer specific enzyme and in the other I used the L- specific form. Assays of each type were in duplicate with a total buffer and reactant volume of 0.8 ml. All biochemicals and buffer constituents used in these assays were purchased from Sigma Biochemicals, St. Louis, Missouri, USA (now Sigma-Aldrich) and were the highest purity available.

I analyzed the other rest and exercise groups (those homogenized in TCA) for anaerobic products using the less specific colorimetric technique of Barker & Summerson (1941) modified for micro samples by Harrower and Brown (1972) and further modified for arachnids by Prestwich (1983a). I analyzed each animal's sample in duplicate with its own lactate-free blank (Prestwich 1983a). This method uses hot acid to generate acetaldehyde from lactic acid; the acetaldehyde then reacts with a dye to produce a color change. Certain other compounds (e.g., some glycolytic intermediates) also react under these conditions to cause color change but they tend to be at low concentration (Barker & Summerson 1941). In earlier work on spiders I found that this method typically gave resting lactate concentrations that were up to 10% higher than found enzymatically, probably as a result of its being less specific (Prestwich 1983). For the present study, I assumed that a discrepancy between colorimetric and enzymatic analysis results for exercised scorpions that approached or exceeded 10% would suggest accumulation of anaerobic by-products in addition to lactate because total amounts of these other compounds would be greater than at rest.

RESULTS

Running.—Figure 1 presents average forced running speeds in body lengths per second with respect to time at 25 °C. These small

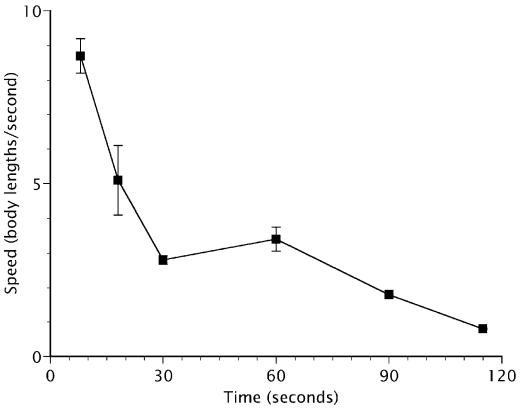

Figure 1.—Running speed in *Centruroides hentzi* during a two min forced activity bout. Speed decreases rapidly over the first 30 s. Error bars indicate 95% confidence intervals. For each point n = 14. Mean body length was 4.7 ± 0.3 cm (SE).

scorpions were surprisingly fast for the first 10 seconds and ran at speeds like those reported by Shaffer & Formanowicz (1996) for a similarly sized species, *C. vittatus* (Say 1821). However within 20 s of the start of exercise, *C. hentzi*'s speed decreased by over 50%; after 30 s, the speed was reduced by nearly 70%. Thereafter, speed reductions were more gradual and some individuals only moved when prodded continuously. Application of the alternative (heat) stimulus (see Methods) at either 30 or 120 s produced no change in activity. This implied that fatigue observed at those times was not the result of habituation to the prod.

Lactate metabolism.—Table 1 presents the results of the different assays for anaerobic products. The non-specific assay (column 1) shows that a small compound or compounds with chemical properties similar or identical to lactic acid is/are present at rest and then accumulate(s) during two mins of forced activity. The compound is not the L(+) optical isomer of lactate (the one found in many animals, including perhaps all chordates). I detected none in two different groups of scorpions that were either resting or exercised for two minutes. When I analyzed these same samples for the D(-) optical isomer, I found this substance in resting scorpions at levels that are statistically indistinguishable from the results of the non-specific test. At the completion of two mins of forced exercise, D(-)-lactate concentration had increased significantly to values about 5.8 times those at rest. The total accumulation of lactate or lactate-mimicking compound(s) in the colorimetric test was about 7% greater than the value found for D-lactate, a statistically significantly difference (ANOVA, P = 0.03, 1,8 *df*) similar to that observed in spiders (Prestwich 1983a).

DISCUSSION

Rapid fatigue during forced running by *C. hentzi* (Fig. 1) is not expected in aerobically fueled activity. In spiders, a similar pattern is associated with accumulation of anaerobic by-products and depletion of arginine phosphate (Prestwich 1983a, b, 1988b). Although there are undercurrents of controversy in the exercise science community, the consensus continues to be that lactate accumulation is associated with fatigue because cells are not fully able to buffer H^+ ions that accumulate when lactate is produced but not sufficiently eliminated (McArdle et al. 2001). This must be especially true in animals that possess limited aerobic capacities and lack highly efficient circulatory mechanisms to move lactate away from muscles to other parts of the body.

Table 1.—Estimates of anaerobic metabolism in the scorpion *Centruroides hentzi*. Sample sizes refer to each treatment. Mean mass and S.E. for each treatment group is given in parentheses.

	Anaerobic Products (μmol/g, $\overline{X}$ ± SEM)		
Condition of scorpion	Colorimetric assay (Non-specific) n = 6	D-(−)-lactate n = 4	L-(+)-lactate n = 4
Rest	0.59 ± 0.003 (0.192 ± 0.010 g)	0.67 ± 0.15 (0.178 ± 0.021 g)	None detected
Two min forced activity	4.15 ± 0.044 (0.181 ± 0.007 g)	3.91 ± 0.07 (0.185 ± 0.015 g)	None detected
Change	3.56	3.34	—

Anaerobic products such as D-lactate are best viewed not as wastes, but instead as storage molecules consisting of an energy-rich carbon skeleton (pyruvate in this case) to which has been added a pair of energy-carrying electrons. These electrons were removed from an earlier glycolytic intermediate. Under conditions where a muscle cell has sufficient aerobic capacity they would instead have been shuttled to the mitochondria to provide an energy source to synthesize ATP. This process can be reversed; lactate can by fully oxidized at a time or place in the scorpion where aerobic conditions exist.

A number of other compounds (e.g., alanine or glycerol-3-phosphate) may potentially serve the same purpose (Prestwich & Ing 1982; Prestwich 1983a). Small accumulations of such compounds could possibly account for the slightly greater concentrations of ''lactate-like'' molecules reported by the colorimetric method as compared to the enzymatic assay (Table 1). However, this difference is small (7%) and could just as well have been due to slight differences in the performances of the individuals that make up the two exercise treatment groups. It is reasonable to conclude that D-lactate is the major if not only anaerobic by product in *C. hentzi* and perhaps all scorpions.

It is unknown why chelicerates produce the D optical isomer of lactate whilst other arthropods, such as crustaceans, produce the L optical isomer and others (most insects) appear to have lost the expression of the LDH gene (Long & Kaplan 1968; Sacktor & Wormser-Shavit 1966). It is possible that the two enzymes may have significant structural differences—arthropod D-LDH has a molecular weight less than half that of L-LDH (Long & Kaplan 1968) but it is also possible that the difference is that arthropod L-LDH is a dimer and D-LDH a monomer and that differences are less than suggested by their apparent molecular weights. There is no evident functional advantage of one version of this enzyme over the other. Both readily reduce pyruvate to lactate, and thereby help to maintain a redox state that allows glycolysis to proceed.

The resting and exhaustion lactate concentrations reported in Table 1 are about half of those measured in spiders (Prestwich 1983a; Anderson & Prestwich 1985), terrestrial crabs (Full & Herreid 1984; Full 1987) and terrestrial ectothermic vertebrates (Bennett 1978). This does not mean that individual active scorpion muscles produce less lactate. The most likely explanation is that the proportion of body mass devoted to running muscles is lower in scorpions than in these comparison groups. Approximately 70% of the total body mass of *C. hentzi* is made up of tissues likely to have no more than minor involvement in running: the opisthosoma, telson and chelapeds. By contrast, in spiders, both leg and prosomal muscles are heavily involved in locomotion (Anderson & Prestwich 1974) and opisthosomal mass was often no more than 40% of total mass (Prestwich 1983a, b).

The extensive use of anaerobic metabolism by intensely active scorpions and spiders may be a consequence of using pressure to extend certain joints and is part of a suite of adaptations that relate to low energy expenditure. In spiders, prosomal pressures as high as 450 torr are generated during struggling, running and jumping and these prevent the circulation of well-oxygenated hemolymph from the book lungs to active muscles because maximum cardiac pressures are below 100 torr (Parry & Brown 1959; Anderson & Prestwich 1974; Prestwich 1988a, b). In scorpions, hemolymph pressures that reach 200 torr are used in conjunction with elasticity to extend certain joints lacking extensor muscles, particularly the chela (Alexander 1967; Sensenig & Shultz 2003, 2004). If hemolymph pressures are high near active muscles, it is reasonable to predict low oxygen availability and reliance on anaerobic metabolism.

Anaerobic metabolism seems to represent an ''inexpensive'' alternative to reliance on aerobic processes for fueling movement. Compared to the aerobic pathway proteins and cytochromes of the mitochondria, relatively few types of proteins need to be produced to support anaerobic metabolism. It must be noted that the energetic advantage of building and maintaining fewer mitochondria is lessened somewhat by the need to synthesize high concentrations of the enzymes required to achieve high rates of anaerobic metabolism. However, anaerobic systems do not require the well-developed circulatory and respiratory system supports needed by highly active cellular aerobic systems. This, in turn, helps to enable a low resting rate of metabolism—a boon to scorpions, spiders, and likely other

exclusively predaceous orders of arachnids that are adapted to irregular and low food availability (Anderson 1970, 1974; Prestwich 1983b; Lighton et al. 2001).

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