Respiration of *Opistophthalmus pugnax* (Thorell) (Scorpionidae) after treadmill exercise and book lung occlusion

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After the four spiracle pairs of *Opistophthalmus pugnax* were stepwise occluded, the oxygen consumption rate ($\dot{M}O_2$) and the accumulation of d-lactate in the haemolymph were measured. D-lactate and $\dot{M}O_2$ was also measured during a 15-minute treadmill exercise at a run speed of 0.025 m/sec. The highest d-lactate concentration of 15 mmol/l was obtained after a 15-minute exercise followed by 11 mmol/l d-lactate when all eight spiracles were closed off for one hour. The $\dot{M}O_2$ value of 1.93 mmol/l obtained from four closed spiracles does not differ significantly ($P < 0.05$, *t*-test) compared with the $\dot{M}O_2$ value of eight open spiracles. Possible mechanisms of metabolic rate depression and metabolic down-regulation are discussed. Low haemolymph pH (7.38 ± 0.08) in *O. latimanus* and other scorpions compared with other terrestrial arthropods are a hallmark inherent to scorpions living in dry xeric conditions.

Key words: spiracle occlusion, oxygen consumption, d-lactate, treadmill exercise.

INTRODUCTION

In some invertebrate groups, d-lactate is produced by cells exposed to anaerobic conditions, as a result of which d-lactate dehydrogenase (d-LDH) is involved in the reduction of pyruvate to d-lactate. In vertebrate groups, however, l-lactate is constantly produced from pyruvate *via* the enzyme l-LDH in a similar process of fermentation. During conditions of exhaustive exercise, sustained exercise or deliberate hypoxia, the rate of d- or l-lactate production exceeds the rate of lactate removal. Increased lactate concentration in blood and cells has been the concern of exercise physiologists and physiological ecologists. They 'used lactate accumulation to estimate maximal sustainable locomotor speeds of animals and to estimate the metabolic and energetic consequences of behaviours in the field as diverse as egg laying, nest building, vocalization, diving, territorial defence and prey capture' (Gleeson 1996). Since the work by Zoond (1931) on the mechanisms and function of the booklungs of the scorpion *Opistophthalmus capensis* (Scorpionidae) measurements on scorpion d-lactate metabolism was done by Prestwich (2006) during maximal running of the scorpion *Centruroides hentzi* (Buthidae). Paul *et al.* (1994a) measured oxygen transport and the physiological role of haemocyanin. Paul *et al.* (1994b) and Dejours & Ar (1991) studied the carbon dioxide transport and acid-base balance in *Pandinus imperator* (Scorpionidae) and *Leiurus quinquestriatus* (Buthidae), respectively. Paul (1991) gave an overview of the gas exchange, circulation, and energy metabolism in arachnids. Interestingly, measurements of the oxygen consumption rate ($\dot{M}O_2$) in scorpions (Lighton *et al.* 2001; Bridges *et al.* 1997; Robertson *et al.* 1982; Hadley 1990; Brownell & Polis 2001) revealed that scorpions in general have low metabolic rates at rest with approximately 25% of the standard metabolic rates compared to other arthropods, especially insects, on a mass-specific basis.

High levels of lactate accumulation in the blood of vertebrates or invertebrates can be accomplished by vigorous muscle exercise. By exposing the cockroach *Periplaneta orientalis* to a rapid stream of hydrogen (Davis & Slater 1928), or by allowing the Weddell seal to dive freely under water (Kooyman *et al.* 1980), similar high concentrations of lactate could be achieved. In the first example the cockroach has to be perfectly quiescent to maintain its resting metabolism while the d-lactate in the body increases in the absence of oxygen. Contrary to this, the Weddell seal is highly active during the dive. In both examples, however, lactic acid accumulation is dependent on a period of absence of oxygen. While l-lactic acid is oxidized by l-LDH in most vertebrates and several other invertebrates (molluscs, crustaceans, polychaets), spiders and scorpions make use of the d-LDH enzyme, exclusively to oxidize d-lactic acid (Long & Kaplan 1969; Critescu *et al.* 2008).

In this study the occlusion of the spiracles of

O. pugnax were again performed as described by Zoond (1931). However, $\dot{M}O_2$ and d-lactate accumulation in the haemolymph was measured after progressive spiracle occlusion. $\dot{M}O_2$ was measured during treadmill exercise. Furthermore, d-lactate concentration data in *O. pugnax* were, on a comparative physiologically basis, compared with other animals under similar anaerobic conditions of metabolism.

MATERIAL AND METHODS

Adult scorpions, 3.2–4.5 g in mass and a body length (from the chelicerae on the mesosoma to the subaculear tubercle of the metasoma (Polis 1990)) between 5.6 and 8.0 cm, were caught at the North-West University's Nooitgedacht recreational farm 17 km southeast of Potchefstroom (26°55′10″S 27°10′5″E). The habitat is typically a rocky, hilly area covered with low scrub and grass. During daytime the scorpions stay in their underground burrows, 20–30 cm deep, made by them. The vertical burrow is usually covered by a flat stone in the rocky soil. Each scorpion was dug out, starting from the typical oval shaped burrow entrance situated alongside the stone. Upon sighting the hiding scorpion, a paint brush was used to expose and clear the animal from the surrounding soil before it was pulled out. Usually three, but not more than five uninjured animals could be caught per day. In the laboratory the scorpions were individually kept in 2-l plastic bottles filled with loose but not dry soil covered with a ceramic tile. During the first night each scorpion usually dug a burrow underneath the tile. They were fed weekly with live grasshoppers or crickets.

The oxygen consumption rate ($\dot{M}O_2$) was measured with a constant volume respirometer (Scholander 1950; Van Aardt 1991) submerged in a water bath at 25 °C. The volume of the respiration chamber could be adjusted to facilitate restricting the body movements of the scorpion inside the chamber. In this confined state the resting or standard metabolic rate was measured. Sodium hydroxide was used to absorb expired CO_2 (Van Aardt 1991). After one hour acclimatization at 25 °C the $\dot{M}O_2$ for each control scorpion ($n = 18$) was determined. Three measurements were made per animal, 30 min apart and the average value taken. One week later, using the 18 control animals, resting $\dot{M}O_2$ measurements were made with six scorpions after closing the two frontal spiracles (1 + 2); with another six animals after closing four spiracles (1+2; 3+4); with another six animals after closing all eight spiracles. The spiracles were occluded using Dunlop rubber solution (Zoond 1931). $\dot{M}O_2$ measurements were also made on a specially designed treadmill (Van Aardt 2010). The six animals were each exercised for 15 min on the treadmill, with a speed fixed at 0.025/msec and the $\dot{M}O_2$ measured during the run.

The d-lactate concentration of the haemolymph was determined for each scorpion after acclimatization at 25 °C for one hour, while confined in the respirometers. Haemolymph was sampled and d-lactic acid analysed. This was done with a specially designed glass collection bulb (0.76 ml capacity) fitted with a syringe needle (outer diameter 0.35 mm) at one end and a silicone tube at the other end (Van Aardt 1991). With mouth suction, between 60 to 80 μl haemolymph could be sampled per animal. The d-lactate was determined for 15 control scorpions with no spiracles occluded; five scorpions with four spiracles occluded and lastly five scorpions with all eight spiracles occluded. The d-lactate analysis was done with an enzymatic method (Boehringer-Mannheim, no. 1112821 at 365 nm using an Eppendorf photometer no. 1101 (Hamburg, Germany) (Van Aardt 2010). The pH of the haemolymph was measured directly after sampling, using a micro-capillary electrode (Type G299, Radiometer, Copenhagen) with a capacity of 12 to 15 μl and a PHM73 pH meter (Radiometer) standardized with Radiometer precision buffer solutions S1510 (pH 7.383) and S1500 (pH 6.841) at 25 °C. Differences between means of data sets were computed using paired *t*-tests at the 95% level of confidence.

RESULTS

Occlusion of all eight spiracles for one hour increased the d-lactate from a value of 0.994 mmol/l without occluded spiracles, to more than 11 mmol/l. When half of the spiracles were closed d-lactate values are about half the value compared with the eight closed spiracles (Table 1). The pH values of the haemolymph decreased significantly ($P < 0.05$, *t*-test) from 7.38 units measured for resting scorpions to 6.78 units when scorpions were exercised for 15 min on the treadmill. Contrary to the d-lactate values the $\dot{M}O_2$ values did not decrease significantly with increasing spiracle occlusions (*t*-test, $P > 0.05$). However, a 150%

Table 1. Effect of spiracle occlusion and treadmill exercise on the haemolymph d-lactate concentration, pH of haemolymph and oxygen consumption rates ($\dot{M}O_2$) of *Opistophthalmus pugnax* at 25 °C; *n* = number of animals; ± = standard deviation of the mean. Occlusion measurements with two book lungs open were not done

Treatment	Haemolymph d-lactate* (mmol/l)	Haemolymph pH*	$\dot{M}O_2$* (µmol/g/h)
8 spiracles open (rested)	[A]0.99 (±0.05; *n* = 15)	[B]7.83 (±0.08; *n* = 5)	[C,E]2.20 (±0.05; *n* = 18)
6 spiracles open (rested)	–	–	[D,E]2.20 (±0.05; *n* = 18)
4 spiracles open (rested)	[A]6.14 (±0.99; *n* = 5)	–	[E]1.93 (±0.05; *n* = 6)
8 spiracles closed (rested)	[A]11.09 (±0.84; *n* = 5)	–	0.0
8 spiracles open (exercised 15 min at 0.025 m/sec)	[A]15.10 (±2.8; *n* = 5)	[B]6.78 (±0.2; *n* = 5)	[F]5.40 (±0.5; *n* = 6)

*t-test ($P < 0.05$): d-lactate values[A] differ significantly from each other.
Haemolymph pH values [B] differ significantly from each other.
$\dot{M}O_2$ values designated as [C] and [D] do not differ significantly.
$\dot{M}O_2$ values at 8 [E]; 6 [E] and 4 [E] differ significantly from exercised scorpions [F].

increase of the $\dot{M}O_2$ values was obtained when the scorpions were exercised for 15 min on the treadmill compared to resting animals with open spiracles (Table 1).

DISCUSSION

When lactate values are compared between species or between different animal groups a sharp difference must be made between exhaustive brief exercise, sustained exercise for considerable periods, and occlusion of the respiratory openings. Prestwich (2006), for example, exercised the scorpion *Centruroides hentzti* for two minutes by forced activity and found less than 4 mmol/l d-lactate in the body while our results showed more than 15 mmol/l d-lactate after 15 sustained minutes of exercise on the treadmill. Similar d-lactate values were reported for the haemolymph of *P. imperator* 10 min after a 3-min activity bout (Paul & Storz 1987; cited in Paul & Fincke (1989) and in Paul (1991)). Considerably lower pH values, compared with our pH values were reported for the yellow scorpion *Leiurus quinquestriatus* by Dejours and Ar (1991). Furthermore, we used only haemolymph samples, contrary to the total body d-lactate sampled by Prestwich (2006) after homogenizing the scorpions. In part this explains the higher d-lactate levels in *O. pugnax* haemolymph. The fact that d-lactate is released from the active muscles into the haemolymph exposes the d-lactate pool to oxidation or use by other tissues in the scorpion body. However, retention of d-lactate inside recovering muscle cells serves to provide for more complete resynthesis of the glycogen stores (Gleeson 1996). Whether the hepatopancreas in *O. pugnax* has glyconeogenesis properties is not clear. However, Srivastava & Kanungo (1966) found that both the Krebs cycle and glycolytic pathway are present in the hepatopancreas of the scorpion *Palamnaeus bengalensis* (Scorpionidae). In comparison with other arthropod animals under similar running conditions in air, the d-lactate in the haemolymph increased above 20 mmol/l in the ghost crab *Ocypode ceratophthalmus* (Van Aardt, 2010) but for the fresh water crab, *Potamonautes warreni*, only 3.75 mmol/l l-lactate was recorded (Adamczewska *et al.* 1997). It may interest comparative physiologists that the highest value of 42.0 mmol/l l-lactate was recorded in the blood of skipjack tuna (Arthur *et al.* 1992) during vigorous exercise.

The $\dot{M}O_2$ values for four out of the eight functional spiracles are only about 12% less compared with when all eight spiracles were functional (Table 1). It shows that the remaining booklungs are capable of compensating for the supply of O_2 when a number of spiracles are occluded. In this regard Millot & Paulian (1943) found that scorpions with only one of the eight spiracles functional, continue to live and feed for several months after the occlusion of the other seven spiracles.

One limitation of closed-system respirometry is that it does not account for activity during measurement. However, the Perspex chambers were observed during measurement to exclude this possibility. The chamber volume could be adjusted to restrict body movements inside the

chamber (Van Aardt 1991). In this confined state the resting or standard metabolic rate was measured. For *O. pugnax,* oxygen consumption rates during rest and during sustainable exercise are well below the oxygen consumption values found for insects with a similar body mass. Oxygen consumption measurements at rest made by Krogh & Weis-Fogh (1951) on the desert locust *Schistocerca gregaria* with an average body mass of 1.8 g, was 21.4 μmol/l/g/h at 25 °C. Compared with the $\dot{M}O_2$ values determined by us (Table 1) on resting *O. pugnax* with a body mass of 3.7 g, the locust uses about 10 times more oxygen on a mass-specific basis. Lighton *et al.* (2001) report that scorpion metabolic rate is roughly 25% that of insects of similar size.

Our oxygen consumption rate results are in accordance with the $\dot{M}O_2$ measurements made by researchers on several scorpion species (Punzo 1991; Prestwich 2006; Lighton *et al.* 2001; Bridges *et al.* 1997; Van Aardt 1991). Low metabolic rates could be ascribed in part to the scorpions' activity behaviour. They are sit-and-wait predators at night, thus conserving energy. During daytime most scorpions stay quiescent in their burrows to preserve energy. More important, with their low metabolic rates and also low heart rates at high temperatures (Bridges *et al.* 1997) their bodies conserve energy during periods of food scarcity, especially in arid and desert environments. Lighton *et al.* (2001) and Milton & Prentice (2007) speculated on the mechanism of metabolic depression and mentioned that unusually low plasma membrane permeability, the reversible down-regulation of voltage-gated potassium channels, high mitochondrial efficiency, and low mitochondrial volume density could be factors involved. Storey (2007), however, pointed out that the molecular basis of metabolic rate depression across phylogeny is a controlled and coordinated suppression of the rates of all ATP-generating and ATP used metabolic functions, resulting in a lower net rate of ATP turnover.

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