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SHORT COMMUNICATION

LACTATE DYNAMICS DURING LOCOMOTOR ACTIVITY IN THE BLUE CRAB, *CALLINECTES SAPIDUS*

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In recent years there has been considerable interest in the effects of locomotor activity on respiratory gas exchange and acid-base balance in the Crustacea (reviewed by Herreid, 1981; McMahon, 1981; McMahon & Wilkens, 1983). However, the partitioning of energy metabolism between aerobic and anaerobic pathways during crustacean locomotion is still poorly understood (Herreid, 1981; Full & Herreid, 1984). Booth, McMahon & Pinder (1982) recently reported that the highly active swimming crab, *Callinectes sapidus*, elevates oxygen uptake rate to a steady state level within 2 min after the onset of enforced swimming activity. In contrast, several terrestrial crabs studied by Herreid and co-workers (Herreid, 1981) showed sluggish aerobic responses to exercise, and did not attain steady state levels of oxygen uptake during 10–20 min of treadmill running at various speeds. The short 'oxygen deficit' period displayed by *C. sapidus* at the start of exercise resembles the oxygen uptake kinetics of highly aerobic mammals (cf. Cerretelli, Pendergast, Paganelli & Rennie, 1979) and suggests that *C. sapidus* might be less dependent on anaerobic metabolism to fuel locomotion than some of the terrestrial crabs studied thus far. Nevertheless, in *C. sapidus* haemolymph lactate concentrations rose to 10 mequiv l^{-1} during 25 min of continuous swimming (Booth *et al.* 1982), indicating that anaerobic glycolysis was utilized to some extent. Because the temporal relationship between lactate formation in the tissues and its appearance in the haemolymph was not known, Booth *et al.* (1982) were unable to determine if lactate was produced only during the 'oxygen deficit' period of early exercise, or if lactate production continued while aerobic metabolism was at steady state. In the present study we report measurements of lactate concentration in haemolymph, locomotor muscle and in the whole animal for *C. sapidus* at rest and at various times during and after enforced swimming activity. These data allow us to examine for the first time in an exercising crustacean the time courses for net lactate formation

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and movement into the haemolymph, as well as the subsequent disappearance of lactate from the tissues and haemolymph during recovery from exercise.

Callinectes sapidus of either sex (mean body mass $112 \pm 7{\cdot}6$ g) were placed in a shallow tank (20 cm water depth) containing a gravel substrate and supplied with flowing sea water (Instant Ocean; 31–32‰ salinity) at 20°C. The crabs were forced to 'swim' for up to 30 min by suspending them in the water and applying tactile stimulation to the carapace. This elicits vigorous beating of the swimming legs (fifth pereiopods) similar to that seen in free-swimming blue crabs (Booth *et al.* 1982). The respiratory and acid-base responses to this type of exercise have been described elsewhere (Booth *et al.* 1982; Booth, McMahon, deFur & Wilkes, 1984).

Haemolymph samples for lactate determination were obtained from submerged crabs by inserting a syringing needle through the arthrodial membrane into the merus of one swimming leg. The haemolymph was immediately deproteinized in ice-cold 12 % (w/v) perchloric acid (PCA) and stored on ice. After the haemolymph sample had been obtained, the crab was quickly removed from the water, the same swimming leg was freeze-clamped by crushing it between aluminium blocks cooled in liquid nitrogen, and the remainder of the animal was immediately homogenized in PCA in a Waring blender. After centrifugation, the haemolymph and whole animal PCA extracts were assayed enzymatically for L-lactate as described by Booth *et al.* (1984).

Approximately 0·1–0·4 g of muscle tissue (abductor and adductor carpopoditus, corresponding to muscles no. 160 and no. 161 of Cochran, 1935) was excised from the merus of the frozen swimming leg, homogenized in PCA and assayed for L-lactate (as above). These muscles, which move the last three segments of the swimming leg towards and away from the median body axis during the swimming stroke, contain both pink and white fibres, but no attempt was made to separate the two fibre types.

Lactate concentrations in the different tissues at rest and during swimming and recovery are shown in Fig. 1. Whole animal [lactate] more than doubled (from 1·2 to 2·9 mequiv kg^{-1} wet tissue; $P<0{\cdot}05$, *t*-test) during the first 2 min of exercise, which corresponds to the 'oxygen deficit' period for this species (Booth *et al.* 1982). Oxygen uptake reached a steady state level within 2 min, yet the production of lactate continued beyond this point, as whole animal [lactate] doubled again (2·9 to 6·0 mequiv kg^{-1}; $P<0{\cdot}05$) between 2 and 10 min. Despite continued vigorous swimming activity there was very little additional lactate accumulation, as whole animal [lactate] rose only to 7·3 mequiv kg^{-1} ($P>0{\cdot}05$) by the end of 30 min.

In resting crabs [lactate] was much higher in swimming leg muscle (8.1 mequiv kg^{-1} wet tissue) than in haemolymph perfusing the muscle (1·1 mequiv l^{-1}) (Fig. 1). With the onset of exercise, the [lactate] gradient between muscle and haemolymph rose sharply, indicating that lactate formation in the muscle exceeded lactate release to the haemolymph. Muscle [lactate] reached a peak of 22 mequiv kg^{-1} at 10 min, and then fell slightly, while haemolymph

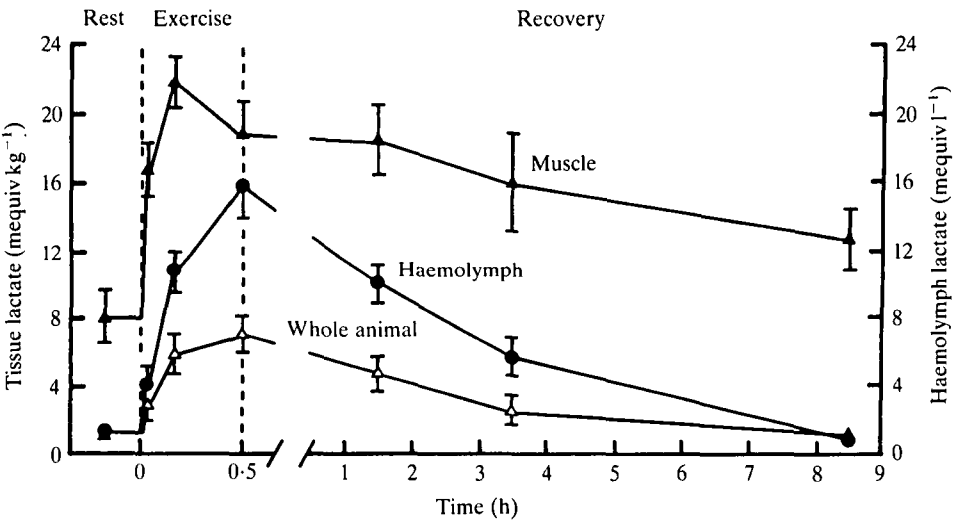

Fig. 1. Lactate concentrations in swimming leg muscle (▲), haemolymph (●) and the whole animal (△) at rest, during swimming activity and during recovery. Mean + S.E. ($N = 5–6$); where standard error bars are not shown the error was smaller than the symbol representing the mean.

[lactate] continued to rise. After 30 min of exercise [lactate] in muscle (18·9 mequiv l^{-1}) and haemolymph (15·8 mequiv l^{-1}) were similar. Since there was little or no net formation of lactate in the animal between 10 and 30 min, most or all of the lactate entering the haemolymph during this period must have been produced in the tissues during the first 10 min of exercise.

Based on the whole animal and haemolymph [lactate] data from Fig. 1, and assuming a haemolymph volume equal to 26 % of body mass (Gleeson & Zubkoff, 1977), it is estimated that the haemolymph space contained 21 % of the total body lactate content at rest and 56 % after 30 min of activity. The release of lactate into the haemolymph during exercise in *C. sapidus* is thus much greater than it is in vertebrates, in which only about 10 % of the total lactate load is ever released into the bloodstream from exercising skeletal muscle (Hermansen & Vaage, 1977; Turner, Wood & Hōbe, 1983). The factors controlling lactate efflux from crustacean muscle are as yet unknown.

During recovery from exercise, whole animal and haemolymph lactate both decreased in an exponential fashion, reaching resting values within 8 h (Fig. 1). At this time only 17 % of the total body lactate content was present in the haemolymph. Muscle [lactate] did not change significantly for the first 3 h of recovery, but by 8 h post-exercise it had fallen to a level not significantly different from the resting value (Fig. 1).

Since oxygen uptake rates were not measured in this study we cannot quantify the contributions of aerobic and anaerobic pathways to total energy metabolism during exercise. Based on the present results plus the oxygen uptake data reported by Booth *et al.* (1982), it appears that anaerobic glycolysis plays an important role in energy production during the first few minutes of swimming activity. Sus-

tained swimming, however, entails a transition to predominantly aerobic metabolism. In contrast, sustained locomotion seems to be fueled largely by anaerobic pathways in a variety of other crab species (Herreid, 1981; Full & Herreid, 1984).

While *C. sapidus* may be less dependent on anaerobic metabolism during sustained locomotion than some other crabs, this does not reflect a lower capacity for performing anaerobic work. Five *C. sapidus* were subjected to a strenuous bout of exercise consisting of vigorous running, struggling and burst swimming until they could no longer right themselves within 15 s after being turned upside down (rested crabs required only 2–3 s to right themselves). The mean time to loss of the righting response was 10·7 min (range 4–15 min), at which point the crabs were clearly fatigued, as they were capable only of slow walking, with little or no movement of the swimming legs. Following this type of activity the mean whole animal [lactate] was 7·7 mequiv kg^{-1}, while haemolymph [lactate] was 11·1 mequiv l^{-1}. The anaerobic capacity for *C. sapidus*, calculated as the maximal increase in whole animal [lactate] after exercise to fatigue (Bennett & Licht, 1972), was 6·5 mequiv kg^{-1}. This value is similar to the anaerobic capacity of the fiddler crab, *Uca pugilator* (6–7 mequiv kg^{-1}; R. J. Full & C. F. Herreid, personal communication), a species which relies on anaerobic metabolism to fuel locomotion (Full & Herreid, 1984), and slightly higher than the anaerobic capacity reported for the shore crab, *Pachygrapsus crassipes* (4·4 mequiv kg^{-1}; Burke, 1979).

During exhausting exercise, *C. sapidus* raises oxygen uptake rate only five-fold over standard aerobic metabolic rate (C. E. Booth & B. R. McMahon, in preparation). This is a rather modest increase compared to the fast-running ghost crab, *Ocypode quadichaudii*, which can raise aerobic metabolic rate by twelve-fold (Full & Herreid, 1983). It thus appears that the blue crab's high speed (Spirito, 1972) and endurance (Fiedler, 1930) locomotor capabilities are related to a well-developed anaerobic capacity plus a rapid shift to aerobic metabolism, rather than to an exceptional aerobic metabolic scope. The mechanisms by which aerobic and anaerobic metabolism are regulated during crustacean locomotion remain unclear. This is an especially interesting area for further study in view of the different metabolic patterns seen among various crab species, and the fact that oxygen transport to the tissues may be modulated by the presence of lactate in the haemolymph (Booth *et al.* 1982).

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