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SHORT COMMUNICATION

URATE DOES NOT ACCUMULATE IN THE HAEMOLYMPH OF EXERCISED BLUE CRABS, *CALLINECTES SAPIDUS*

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L-Lactate, the only known anaerobic end-product in decapod crustaceans (Gäde, 1983), increases haemocyanin oxygen-affinity (Truchot, 1980; Mangum, 1983; Bridges and Morris, 1986). In exercised blue crabs, *Callinectes sapidus*, the decrease in haemocyanin oxygen-affinity induced by metabolic acidosis *via* the Bohr shift was substantially balanced by the opposing effect of L-lactate (Booth *et al.* 1982). Since this study, Morris *et al.* (1985) have discovered that urate also enhances haemocyanin oxygen-affinity in the crayfish *Austropotamobius pallipes*. The importance of urate in regulating oxygen transport during environmental hypoxia has recently been stressed in the crab *Carcinus maenas* and the prawn *Penaeus japonicus* (Lallier and Truchot, 1989*a*,*b*). Since *Callinectes sapidus* haemocyanin exhibits a urate effect (De Fur *et al.* 1990), urate may also play a role during functional anaerobiosis.

In the land crab *Birgus latro*, which is mostly uricotelic (Greenaway and Morris, 1989), there is no increase in haemolymph urate concentration following exercise (Greenaway *et al.* 1988). Because aquatic crustaceans are ammonotelic animals, little attention has been devoted to variations in urate levels in this group. However, it has recently been shown that urate accumulates in the haemolymph of decapod crustaceans exposed to environmental hypoxia (Lallier *et al.* 1987; Czietrich *et al.* 1987; De Fur *et al.* 1990) or elevated temperature (Lallier *et al.* 1987). Its probable origin, even though not directly demonstrated *in vivo*, may be the degradation of purine nucleotide compounds, such as AMP, GMP or IMP, *via* the purinolytic pathway. Xanthine dehydrogenase (XDH; EC 1.2.1.37), the enzyme required to produce urate *via* xanthine from these substrates, is present in the hepatopancreas of both *Carcinus maenas* (Dykens and Shick, 1988) and *Callinectes sapidus* [maximum activity 17.5±7.2 nmol of urate produced per minute and per gram hepatopancreas (mean±S.E., N=7), F. Lallier and P. J. Walsh, unpublished results]. Alternatively, xanthine oxidase (XOD, EC 1.2.3.2), which uses oxygen rather than NAD^+ as a cofactor, may play the role of XDH.

Key words: urate, exercise, oxygen transport, Crustacea, *Callinectes sapidus*.

Of these two enzymes, XOD is the only one present in the hepatopancreas of *Birgus latro* (Greenaway and Morris, 1989), but in *Callinectes* hepatopancreas its maximum activity is lower than that of XDH (3.4 ± 0.7 nmol min^{-1} g^{-1}, F. Lallier and P. J. Walsh, unpublished results).

In addition, recent data on vertebrates suggest that during stress, such as vigorous muscular exercise, the energetic balance may be partly preserved by converting AMP, produced by the breakdown of ATP, into IMP (Meyer and Terjung, 1979; Mommsen and Hochachka, 1988). An analogous mechanism could also be achieved in crabs by turning AMP into urate and releasing it into the haemolymph. As a direct consequence of this hypothesized intracellular regulation process, urate could contribute with L-lactate to the adjustment of oxygen binding to haemocyanin during exercise. In the present study, we have investigated urate accumulation in the haemolymph of blue crabs after exhaustive exercise.

Blue crabs (five females, wet mass 80–200 g) were purchased from Gulf Specimen Co. (Panacea, Florida) and kept in aquaria with running sea water (approx. 24 °C, 36 ‰). They were fed two or three times a week with chopped frozen shrimp or squid, until 24 h before an experiment.

The experiment was designed as randomized blocks on five individuals, the fixed factor being the time of sampling: before exercise (control), immediately after a 30-min exercise session, or following a 2-h recovery period. Control and recovery conditions were well-aerated flowing sea water (24 °C, 36 ‰). Exercise consisted of exhaustive muscular activity: suspending the crab in the water column initiated continuous movements of the swimming legs. The crabs were also stimulated by tactile stimulations towards the end of the 30 min.

Samples of prebranchial haemolymph (0.3–0.4 ml) were withdrawn from the infrabranchial sinus using a hypodermic needle (G22). Blood collected in 1 ml plastic syringes was immediately transferred into ice-cold microcentrifuge tubes kept on ice, and a 0.1 ml subsample was immediately deproteinized in 0.2 ml of 8 % (v/v) perchloric acid. We also measured lactate and haemocyanin levels. Lactate was used as a test of the exercise protocol and for comparative purposes, and haemocyanin concentration served as a validation that repetitive sampling did not adversely affect the haemolymph volume or water content.

Lactate was assayed enzymatically with a Boehringer Mannheim test kit (catalogue no. 139084) on the subsamples of deproteinized haemolymph. Urate concentrations were determined by an enzymatic assay (Sigma procedure no. 685) using a correction for haemocyanin absorbance (see Lallier and Truchot, 1989*b*). The difference in absorbance at 335 nm of diluted (1:20 in normal crab Ringer) haemolymph before and after addition of Na_2SO_3 was used to calculate haemocyanin concentrations using $A^{1\%}_{1cm}=2.23$ (Nickerson and Van Holde, 1971). Each measurement was performed in duplicate and the mean taken as an individual value.

Haemocyanin concentrations demonstrated a slight, non-significant decrease, consistent with the repetitive sampling of haemolymph (three times 0.3–0.4 ml,

Table 1. *Urate, L-lactate and haemocyanin concentrations in the haemolymph of blue crabs sampled at rest (control), after 30 min of exercise and after 2 h of recovery*

	Control	Exercise	Recovery
Urate ($mmol\,l^{-1}$)	0.101±0.017	0.090±0.014	0.061±0.004
L-Lactate ($mmol\,l^{-1}$)	0.46±0.09	13.78±0.78**	6.04±0.79*
Haemocyanin ($g\,l^{-1}$)	41.5±4.2	40.7±2.1	35.9±2.1

Temperature 24°C; salinity 36‰.

Values are mean±S.E. (N=5), and symbols give the result of comparison with control values using paired t-test: * $P<0.01$ and ** $P<0.001$. Other comparisons were not significant.

Table 1). Values were typical of intermoult blue crabs acclimated to full-strength sea water (Horn and Kerr, 1969).

As expected, the intense muscular activity induced a marked elevation of lactate concentration (Table 1), well within the range of values reported for this species (e.g. Booth *et al.* 1982, 1984; Booth and McMahon, 1985; Milligan *et al.* 1989). After 2 h of recovery, lactate concentration decreased ($P<0.01$), but it was still significantly higher than the resting value ($P<0.01$). Again, these changes in the concentration of lactate are in agreement with values existing in the literature; complete recovery of resting lactate concentration values may take as long as 8–12 h (Bridges and Brand, 1980; Booth *et al.* 1982, 1984; Ellington, 1983; Milligan *et al.* 1989).

Urate concentrations did not change significantly during exercise or recovery (Table 1, two-tailed paired t-test, $P>0.05$). The slight apparent decrease of urate concentration may be correlated with the concomitant decrease of haemocyanin concentration as urate binds to the pigment (Lallier, 1988). The absence of significant changes in haemolymph urate concentration indicates that urate cannot be an important modulator of haemocyanin function during exercise in *C. sapidus*.

Why does urate concentration increase during hypoxia but not after strenuous exercise? Putative mechanisms of urate accumulation during hypoxic exposure involve its production by XDH, whose activity in *Carcinus maenas* hepatopancreas is sufficient to explain the observed *in vivo* increase in urate concentration (Lallier *et al.* 1987; Dykens and Shick, 1988), and an inhibition of degradation of urate by uricase, an oxygen-requiring enzyme (M. K. Grieshaber, personal communication). In contrast with hypoxia, postbranchial P_{O_2} remains high during muscular exercise (e.g. Booth *et al.* 1984), and it is possible that oxygen levels in the hepatopancreas remain high enough to continue to oxidize urate to allantoin. However, this is speculative and other sources of urate may be involved (e.g. *de novo* purine synthesis, as suggested in the land crab *Birgus latro* by Greenaway and Morris, 1989). Clearly, the precise mechanisms that are responsible for urate production or breakdown, together with their regulation under stress conditions, should be further investigated at the cellular level.

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