

POST-EXERCISE LACTATE PRODUCTION AND METABOLISM IN THREE SPECIES OF AQUATIC AND TERRESTRIAL DECAPOD CRUSTACEANS

RAYMOND P. HENRY

*Department of Zoology and Wildlife Science and the Alabama Agricultural Experiment
Station, 101 Cary Hall, Auburn University, Auburn, AL 36849-5414, USA*

CHARLES E. BOOTH

*Department of Biology, Eastern Connecticut State University, Willimantic,
CT 06226, USA*

FRANCOIS H. LALLIER* AND PATRICK J. WALSH

*Division of Marine Biology and Fisheries, Rosenstiel School of Marine and Atmospheric
Science, 4600 Rickenbacker Causeway, Miami, FL 33149-1098, USA*

Accepted 8 September 1993

Summary

Aquatic and terrestrial crustaceans are dependent on both aerobic and anaerobic metabolism for energy production during exercise. Anaerobic energy production is marked by an accumulation of lactate in both muscle tissue and haemolymph, but the metabolic fate of lactate is not clear. Lactate recycling *via* gluconeogenesis and the potential role of carbonic anhydrase (CA) in supplying bicarbonate for the carboxylation of pyruvate were investigated in three species of decapod crustaceans: *Callinectes sapidus* (aquatic), *Cardisoma guanhumi* (semi-terrestrial) and *Gecarcinus lateralis* (terrestrial). CA activity was found in mitochondria and cytoplasmic fractions of gill, hepatopancreas and muscle of all three species. Significant activities of key enzymes of gluconeogenesis (e.g. pyruvate carboxylase, phosphoenolpyruvate carboxykinase and fructose biphosphatase), however, could not be detected. Exercise to exhaustion produced a species-specific pattern of accumulation and clearance of lactate in tissue and haemolymph, indicating a differential degree of reliance on anaerobic energy production. Treatment with acetazolamide, a CA inhibitor, did not significantly alter the pattern of lactate dynamics in animals given repeated bouts of exhaustive exercise interspersed with periods of recovery. Injection of [U-¹⁴C]lactate resulted in the appearance of label in both muscle glycogen and excreted carbon dioxide, suggesting multiple metabolic fates for lactate. Lactate turnover rates for *G. lateralis* were similar to those reported for fish. In these animals, gluconeogenesis possibly proceeds *via* the reversal of pyruvate kinase, or *via* the typical Cori cycle but so slowly that the uncatalysed supply of bicarbonate is

*Present address: Laboratoire de Biologie Marine, Université Pierre et Marie Curie, 75252 Paris, Cedex 05, France.

Key words: exercise, lactate, crabs, carbonic anhydrase, *Callinectes sapidus*, *Cardisoma guanhumi*, *Gecarcinus lateralis*.

sufficient to keep pace with the low activities of pyruvate carboxylase and the subsequent low rates of pyruvate carboxylation.

Introduction

Many vertebrate and invertebrate species rely at least partly on anaerobic energy production during periods of exercise. In crustaceans, reliance on anaerobic metabolism during strenuous bouts of exercise is usually characterized by the degree of lactate accumulation in the haemolymph (e.g. from resting values below 1 mmol l^{-1} to post-exercise values of between 2 and 25 mmol l^{-1}) (McDonald *et al.* 1979; Smatresk *et al.* 1979; Wood and Randall, 1981; Booth *et al.* 1984; Forster *et al.* 1989; Morris and Greenaway, 1989). Lactate is the primary metabolic acid produced in decapod crustaceans and it constitutes virtually the total metabolic component of exercise-induced acidosis (Wood and Randall, 1981; McDonald *et al.* 1979).

While the acid–base consequences of post-exercise lactate accumulation have been well documented, much less is known about the metabolic fate of lactate and the pathways of lactate metabolism in crustaceans. In mammals, a significant fraction of lactate is remetabolized to glycogen *via* the Cori cycle (gluconeogenesis) in the liver and the kidney (reviewed by Hers and Hue, 1983). Among lower vertebrates, the situation is somewhat different. Muscle fibres in reptiles appear to be a significant site of gluconeogenesis, utilizing lactate as a substrate for post-exercise glycogen restoration (Gleeson and Dalessio, 1989, 1990). In fish, the liver, kidney and muscle have all been implicated as potential sites of gluconeogenesis (Suarez and Mommsen, 1987; Milligan and McDonald, 1988; Walsh, 1989).

In crustaceans, the fate of lactate and the potential role of gluconeogenesis is much less clear. Injections of [^{14}C]lactate have shown, based solely on the accumulation of radioactivity in glycogen in haemolymph, muscle and hepatopancreas, that crustaceans appear to have gluconeogenic capability (Thabrew *et al.* 1971; Gade *et al.* 1986; van Aardt, 1988). The major enzymes of the gluconeogenic pathway, however, have never been completely characterized in crustaceans. Thabrew *et al.* (1971) reported activities for glucose-6-phosphatase (G-6-Pase) and fructose-1,6-bisphosphatase (FBPase) in the gills of *Carcinus maenas*, but they did not assay other enzymes or other tissues. In several species of crabs, phosphoenolpyruvate carboxykinase (PEPCK) and FBPase activities were very low in all tissues, and pyruvate carboxylase (PC) was absent, while the hepatopancreas had virtually no detectable lactate dehydrogenase (LDH) activity (Walsh and Henry, 1990; Lallier and Walsh, 1991a).

Additionally, in a recent study, isolated hepatopancreas cells from the blue crab have been shown to account for only 1 % of the post-exercise processing of lactate (Lallier and Walsh, 1992). Thus, the gluconeogenic capability, location and pathway in crustaceans is still uncertain.

In addition to the traditional enzymes of the Cori cycle, recent evidence has been presented suggesting that the enzyme carbonic anhydrase (CA) plays a critical role in lactate recycling among vertebrates by supplying HCO_3^- , through the catalyzed hydration of CO_2 , for the carboxylation of pyruvate (Herbert *et al.* 1983; Dodgson and

Forster, 1986). In mammals, the CA V isoenzyme is known to be co-localized intramitochondrially with PC, but among invertebrates the subcellular distribution of CA in tissues with gluconeogenic potential and the putative role of CA in the process are unknown.

This study was undertaken to compare the relative reliance on anaerobic metabolism during exercise, to clarify the role of gluconeogenesis in post-exercise lactate metabolism and to investigate the potential role of CA in the gluconeogenic pathway in three species of aquatic and terrestrial decapod crustaceans.

Materials and methods

Collection and maintenance of animals

Adult intermoult male blue crabs, *Callinectes sapidus* (Rathbun), were obtained from Gulf Specimen Co., Panacea, Florida. Animals were air-shipped to Miami where they were held in running seawater aquaria (32‰ salinity, 24 °C). Amphibious marsh crabs, *Cardisoma guanhumi* (Latreille), were collected from Parrot Jungle and Gardens, Miami, Florida. They were kept in 60 l terraria at room temperature (approximately 23 °C) with a supply of drinking water (10‰). Terrestrial crabs, *Gecarcinus lateralis* (Fremenville), were obtained from the Bermuda Biological Station for Research, Bermuda; animals were air-shipped to Miami and maintained in sand-filled terraria at room temperature with a supply of drinking water (10‰). All animals were fed daily but were starved for 24 h prior to being used in an experiment.

Enzyme assays

Mitochondria and cytoplasmic fractions were prepared according to standard methods (e.g. Ballantyne and Storey, 1984). Animals were killed, and gill, muscle (white backfin muscle of blue crab, leg muscle of the other two species) and hepatopancreas (approximately 1 g each) were dissected out and placed in five volumes of cold buffer [50 mmol l⁻¹ imidazole, pH 7.0, 0.1 mmol l⁻¹ phenylmethylsulphonyl fluoride (PMSF), made isotonic to the haemolymph of the individual crab species using sucrose]. Tissues were homogenized by 2–5 passes of a Teflon–glass homogenizer powered by an electric drill. The crude homogenate was centrifuged at 250 g for 20 min at 4 °C (Jouan model CR 412). The supernatant was saved and centrifuged at 7500 g for 20 min at 4 °C (Sorvall RC-5B); the resulting pellet (mitochondria) and supernatant (cytoplasm) were saved for enzyme analysis. The mitochondria were resuspended in 1 ml of homogenization buffer and sonicated for 30 s at 25 W (Heat Systems Ultrasonics).

Gluconeogenic enzymes (PC, PEPCK and FBPase) were assayed spectrophotometrically in the cytoplasmic and mitochondrial fractions according to the methods of Mommsen *et al.* (1980) as modified by Walsh and Henry (1990). The enzymes LDH and citrate synthetase (CS) were used as cytoplasmic and mitochondrial markers, respectively, to ensure low cross-contamination between fractions. Carbonic anhydrase was assayed electrometrically according to the method of Henry and Kormanik (1985) as described in detail by Henry (1991).

Exercise to exhaustion

The crabs were subjected to vigorous bouts of exercise that were initiated and maintained *via* tactile stimulation. *C. guanhumi* and *G. lateralis* were exercised by forced walking (Smatresk *et al.* 1979); haemolymph samples (50 μ l) were withdrawn every 5 min over a 20 min exercise period and assayed for lactate. The samples were deproteinized in 100 μ l of cold 8% perchloric acid and stored on ice. Lactate was determined spectrophotometrically (Sigma diagnostics no. 726-UV/826-UV) in the presence of 10 mmol l⁻¹ EDTA to reduce interference by copper. *C. sapidus* were exercised for 60 min by forcing them to swim inside 10 l rubber basins (Booth *et al.* 1982). The water in the basins was partially changed every 5 min to minimize the build-up of excretory products. Haemolymph samples were taken every 10 min and assayed as above. Previous reports have shown that *C. carnifex* (Wood and Randall, 1981) and *G. lateralis* (Smatresk *et al.* 1979) reach exhaustion after 10 min of vigorous exercise. Longer exercise periods were used for *C. sapidus* because of previous reports documenting their resistance to fatigue (Booth *et al.* 1984). These results were used to confirm that the exercise regimes used in this study produced exhaustive conditions for all three species as characterized by near-maximal haemolymph lactate concentrations (see Results). In all subsequent experiments, the exercise regimes were as follows: 20 min for *C. sapidus* and 10 min for *C. guanhumi* and *G. lateralis*.

Lactate accumulation and clearance

Twelve hours prior to an experiment, a small hole was drilled in the dorsal carapace of each crab over the pericardial cavity, and a rubber septum was glued over the hole with cyanoacrylate glue. Animals were then given either an injection of Millipore-filtered sea water made isosmotic to the haemolymph (controls) or an injection of acetazolamide, a potent CA inhibitor. The extracellular fluid (ECF) volume of the crab was estimated to be one-third of the total mass (Gleeson and Zubkoff, 1977), and a volume of a stock solution of acetazolamide (100 mmol l⁻¹) was injected to produce a circulating ECF concentration of 1 mmol l⁻¹. Previous work (Henry and Cameron, 1983; Henry, 1987) has shown that 100 μ mol l⁻¹ acetazolamide permeates gill tissue within 4–6 h and completely inhibits intracellular CA activity for 24 h. In the present experiments, measurements were taken between 12 and 24 h post-injection, a period that corresponds to maximal CA inhibition by the drug.

Crabs were then exercised to exhaustion based on peak haemolymph lactate build-up (i.e. 20 min for *C. sapidus* and 10 min for *C. guanhumi* and *G. lateralis*, see above). Prior to and at various intervals following the exercise periods, haemolymph samples (50 μ l) were withdrawn from the infrabranchial sinus at the base of the walking legs for lactate analysis (see above).

A separate group of crabs was also exercised to exhaustion. Prior to and following the exercise periods, individuals from this group were immediately freeze-clamped by immersion in liquid nitrogen (5 l). Freezing was judged to be complete when the liquid nitrogen ceased boiling (usually within 20 s to 1 min, depending on the size of the crab). Individual tissues were then dissected out, while the crab was still frozen, by fracturing

the carcass with a hammer and chisel. Tissues separated from one another cleanly along the fracture lines, and we were able to obtain good samples of both red and white levator muscle (backfin) for *C. sapidus*, leg muscle from the other two species, and gill and hepatopancreas from all three. These samples were cleaned of shell pieces and minor tissue contamination while still frozen, by scraping with a jeweller's screwdriver, and they were stored at -80°C . The tissues were later thawed to 0°C and homogenized in 8% perchloric acid (PCA) (approximately 0.25 g in 1 ml), centrifuged at 13 000 g (20 min at 5°C), and assayed for lactate as above. Tissue homogenates were also assayed for glycogen (Keppler and Decker, 1984) and glucose (Sigma diagnostics no. 16 uV) concentrations.

Acetazolamide inhibition of carbonic anhydrase activity

In order to test the role of CA in gluconeogenesis, the approach of Herbert *et al.* (1983) was used. Crabs were given repeated bouts of exhaustive exercise separated by a recovery period, during which tissue glycogen concentrations were allowed to be restored. Crabs were exercised after an injection of either saline (control) or acetazolamide (given 12 h prior to the first exercise period to produce maximal CA inhibition). Haemolymph samples ($50\ \mu\text{l}$) were taken throughout the experiment for lactate and glucose analysis. All animals were monitored for 24 h after the end of an experiment, and only the data from individuals that survived through that period were used.

In another group of crabs, resting and post-exercise individuals were freeze-clamped in liquid nitrogen as above, and gill, hepatopancreas and muscle tissue were dissected out and homogenized in $0.6\ \text{mol l}^{-1}$ PCA. Tissue lactate, glycogen and glucose concentrations were determined as described above.

Lactate turnover and glycogen restoration rates

The measurement of lactate turnover rates was attempted using radioactive tracer methods similar to those employed for fish (Milligan and McDonald, 1988). Crabs were injected through the rubber septum above the heart with a stock solution of L-[U- ^{14}C]lactate in saline ($0.25\ \text{ml}\ 100\ \text{g}^{-1}$ body mass of $20\ \mu\text{Ci ml}^{-1}$ solution) at 1 h post-exercise. A series of haemolymph samples ($50\ \mu\text{l}$) was taken from the infrabranchial sinuses as above at 1.5, 3, 4, 5, 7.5, 10, 12.5, 15, 17.5, 20, 30 and 60 min following the injection (i.e. between 1 and 2 h post-exercise). The difference in injection and sampling sites allowed for complete mixing by the time the first sample had been taken. Samples were added to $100\ \mu\text{l}$ of 70% PCA and stored at 4°C until analysis. Lactate specific activity in haemolymph was determined by the method of Roca *et al.* (1985); total lactate was determined as above. Where possible, lactate turnover values were calculated according to Milligan and McDonald (1988) by integrating the area under the curve of lactate specific activity ($\text{disints min}^{-1}\ \mu\text{mol}^{-1}$) versus time from time zero to the time at which specific activity equalled 5% of the initial specific activity (calculated by extrapolating the fitted curve back to $t=0$).

At 2 h post-exercise, the crabs were killed, and muscle was harvested and frozen as above. For *C. sapidus*, both red and white levator muscle were sampled. Total glycogen and radioactivity incorporated into muscle glycogen were determined by purifying

glycogen by the methods of Johnson and Fusaro (1966). Ecolume (ICN) was the fluor in both cases, and samples were counted by liquid scintillation (Tracor Analytic). Mean total glycogen synthesis rates were calculated using the mean haemolymph lactate specific activity for the 1 h period between 1 and 2 h post-exercise (i.e. the 30 min haemolymph sample), the specific activity of muscle glycogen and the proportion of body mass that is muscle for each species (determined empirically). Additionally, for blue crabs, for part of the incubation period, water samples were taken and counted prior to and after acid treatment (to release CO_2). Terrestrial crabs were incubated in a sealed respirometer with filter paper soaked in hyamine hydroxide to trap $^{14}\text{CO}_2$ (trapping efficiency of greater than 95%); at the end of the experiment, the filter paper was also counted for ^{14}C radioactivity.

Results

Comparative effects of exercise

The exercise periods appeared to produce peak haemolymph lactate concentrations in each species (i.e. 85–90% of maximum measured values) (Fig. 1). Qualitative observations on the behaviour of the crabs during and after exercise, however, indicated differences in the species' abilities to perform sustained vigorous exercise. *C. sapidus* maintained a high degree of activity, was continually responsive to tactile stimulation, and was never fatigued to the point of becoming refractory. Also, haemolymph lactate concentrations did not begin to level off until after 20 min (Fig. 1). The other two species were much less resistant to fatigue. *C. guanhumi* were able to exercise vigorously (i.e. sprint) for about 4 min, after which they became progressively slower and refractory (i.e.

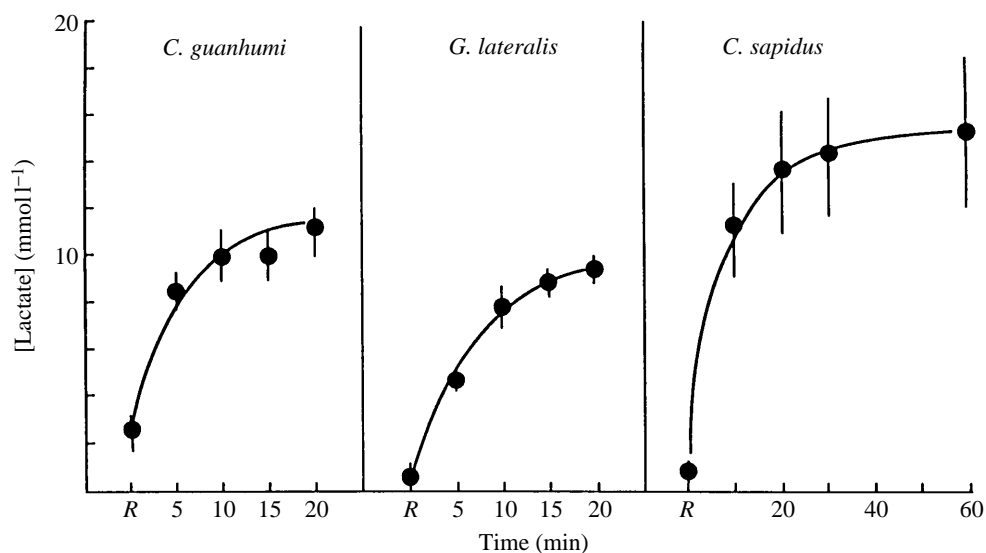


Fig. 1. Haemolymph lactate concentrations at rest (R) and during a single bout of prolonged exhaustive exercise in three species of crabs. Mean \pm S.E.M. ($N=5$). $t=23^\circ\text{C}$.

Table 1. Carbonic anhydrase activities in the mitochondrial and cytoplasmic fractions of gill, hepatopancreas and muscle of three species of decapod crustaceans

Species	Tissue	Carbonic anhydrase activity ($\mu\text{mol CO}_2 \text{g}^{-1} \text{min}^{-1}$)	
		Cytoplasm	Mitochondria
<i>Callinectes sapidus</i> (N=6)	Gill	4922±889	7944±1370
	Hepatopancreas	3565±871	2474±480
	Muscle	4144±1362	2261±1090
<i>Cardisoma guanhumi</i> (N=4)	Gill	165 445±17 692	83 200±18 572
	Hepatopancreas	553±146	529±157
	Muscle	1739±423	1273±314
<i>Gecarcinus lateralis</i> (N=4)	Gill	43 213±15 994	50 063±10 700
	Hepatopancreas	100 024±20 338	32 908±4645
	Muscle	2967±1048	2666±582

Mean \pm S.E.M. (N=4–6), $t=25^\circ\text{C}$.

animals sat with their abdomens touching the ground, their chelae raised in the air, and were not responsive to tactile stimuli). Haemolymph lactate concentrations also peaked early (5 min) and did not change thereafter (Fig. 1). *G. lateralis* were only able to maintain vigorous exercise for 1–2 min; after that they could not be stimulated to move at more than a slow steady pace, usually becoming refractory before the end of the exercise period.

Enzyme activities and distribution

The distribution of LDH and CS indicated little contamination of either the cytoplasmic or mitochondrial fractions (results not shown). Carbonic anhydrase activity was detected in both fractions of gill, in the hepatopancreas and in the muscle of all three species (Table 1), showing its typical ubiquitous presence (for reviews, see Maren, 1967; Henry, 1984).

The enzymes of gluconeogenesis that were assayed (PC, PEPCK and FBPase) were not detected in any significant levels of activity in either the mitochondria or cytoplasm of any tissue in any of the three species. These results agree with previous studies on *C. sapidus*, in which very low enzyme activities were detected in crude unfractionated homogenates (Walsh and Henry, 1990; Lallier and Walsh, 1991b).

Lactate accumulation and clearance

A single bout of exercise produced peak haemolymph lactate concentrations of 9–10 mmol l^{-1} in both *C. sapidus* and *G. lateralis* between 0 and 1 h post-exercise (Fig. 2). The values for *C. sapidus* are about one-third lower than those reported by Booth *et al.* (1984) for animals that had been exercised for 1 h, while the peak lactate concentration in *G. lateralis* was nearly fivefold higher than values reported by Smatresk *et al.* (1979) for identical exercise conditions. In *C. guanhumi*, the peak lactate concentration at 16 $\mu\text{mol l}^{-1}$ was significantly higher than peak values for the other two

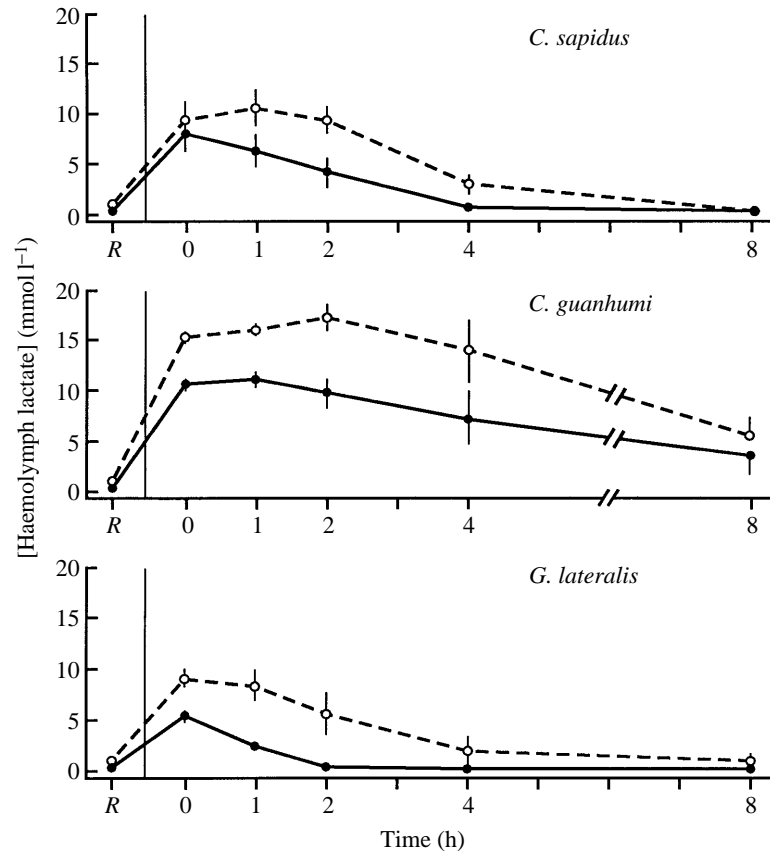


Fig. 2. Haemolymph lactate concentrations at rest (*R*) and during recovery after a single bout of exhaustive exercise in three species of decapod crustaceans. Dashed lines and open circles represent control values, solid lines and filled circles represent animals injected with acetazolamide (1 mmol l^{-1} circulating concentration in haemolymph) 12 h prior to an experiment. Vertical bars indicate the exercise period (20 min for *C. sapidus* and 10 min for *C. guanhumi* and *G. lateralis*). Mean \pm S.E.M. ($N=5$). $t=25^\circ\text{C}$.

species ($P<0.05$, *t*-test) and the level remained higher for longer than in the other species, lasting until 4 h post-exercise (Fig. 2). A related species, *C. carnifex*, after 10 min of severe exercise, had maximum haemolymph lactate concentrations only half of those reported here (Wood and Randall, 1981). In *C. sapidus* and *G. lateralis*, haemolymph lactate concentrations returned to near resting values by 4 h post-exercise, but in *C. guanhumi* resting values were not restored until 24 h (Fig. 2).

Treatment with acetazolamide 12 h prior to exercise reduced the peak haemolymph lactate concentration immediately after exercise and during the initial period of recovery in all three species (at 1 and 2 h post-exercises in *C. sapidus*, and at 0, 1 and 2 h post-exercise in the other two species, $P<0.05$, *t*-test). Acetazolamide did not, however, change the rate at which resting lactate concentrations were restored.

Table 2. Lactate and glycogen concentrations in muscle tissue of three species of decapod crustaceans at rest and immediately after a single bout of exhaustive exercise (T0)

Species	Lactate ($\mu\text{mol g}^{-1}$)		Glycogen ($\mu\text{mol glucosyl units g}^{-1}$)	
	Rest	T0	Rest	T0
<i>Callinectes sapidus</i>				
Red levator	1.94±0.45	12.7±0.56	87.3±4.9	48.9±9.9
White levator	1.33±0.50	13.9±1.3	53.0±2.8	24.1±5.8
<i>Cardisoma guanhumi</i>				
Leg	1.56±0.24	15.3±3.5	162.2±16	130.5±11
<i>Gecarcinus lateralis</i>				
Leg	0.63±0.18	8.6±1.3	88.7±6.2	59.8±3.6

Mean ± S.E.M. (N=5), t=25 °C.

Resting and post-exercise lactate concentrations in muscle were also dependent upon the species. At rest, *C. sapidus* and *C. guanhumi* had between 1 and 2 $\mu\text{mol g}^{-1}$ regardless of muscle type, while values in *G. lateralis* were significantly lower than in either of the other two species ($P < 0.05$, *t*-test) (Table 2). Muscle lactate concentrations immediately post-exercise were between 12 and 15 $\mu\text{mol g}^{-1}$ for *C. sapidus* and *C. guanhumi* and were not significantly different ($P > 0.05$, *t*-test) (Table 2). Post-exercise values for *G. lateralis* were significantly lower than for both other species ($P < 0.05$, *t*-test).

Lactate and glucose dynamics during multiple exercise regimes

Inhibition of CA activity by acetazolamide had no significant effect on haemolymph lactate or glucose concentrations in crabs subjected to repeated bouts of exercise. The same depression of haemolymph lactate level was observed in the initial exercise period but, following a recovery period (6–24 h, depending on species), there was no difference in haemolymph lactate concentration between control and drug-treated animals after the second exercise period in any species (Fig. 3). This result contrasts with those from similar experiments performed on lower vertebrates (Herbert *et al.* 1983) and suggests that, in crabs, CA inhibition does not retard glycogen replenishment. Furthermore, acetazolamide did not eliminate haemolymph hyperglycaemia after repeated bouts of exercise (Fig. 4), again in contrast to the inhibitor's effects on lower vertebrates (Herbert *et al.* 1983).

Muscle lactate and glycogen

Resting glycogen concentrations are both tissue- and species-specific. For *C. sapidus*, red muscle had significantly higher glycogen concentrations at rest and after exercise than did white muscle ($P < 0.05$, *t*-test; Table 2). Leg muscle of *C. guanhumi* had roughly twice as much glycogen as that of *G. lateralis* (Table 2).

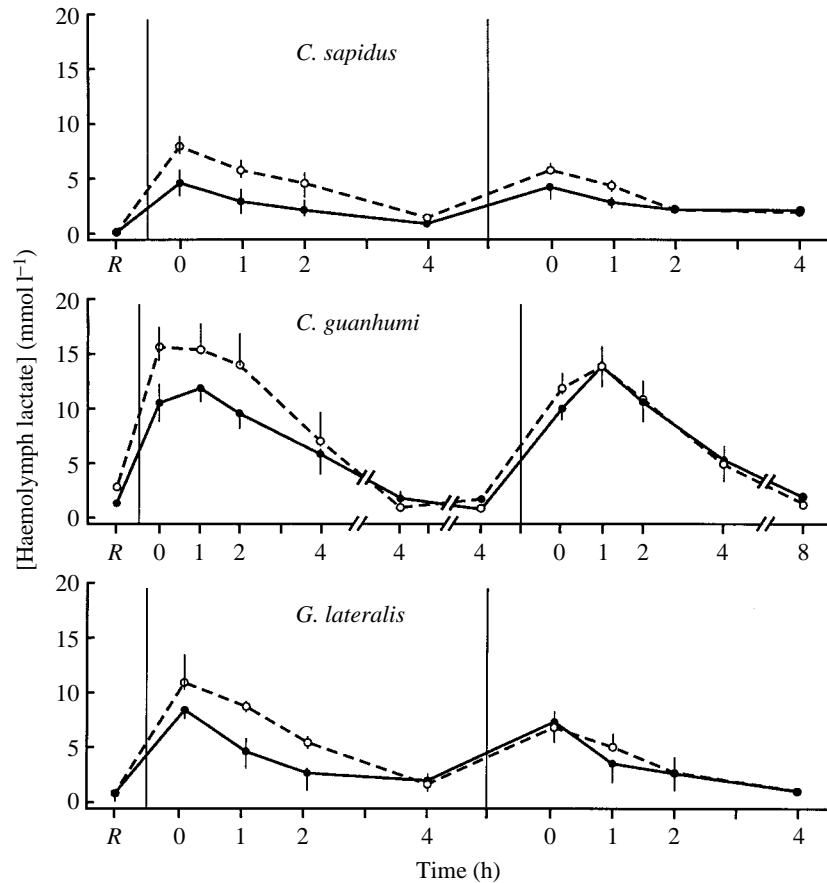


Fig. 3. Haemolymph lactate concentrations at rest (*R*) and during recovery after two successive bouts of exhaustive exercise in three species of decapod crustaceans. Dashed and solid lines and vertical bars as in Fig. 2. Mean \pm S.E.M. ($N=5$). $t=25^{\circ}\text{C}$.

For *C. sapidus*, there were no differences in post-exercise peak lactate concentrations between red and white muscle, and between control and acetazolamide-treated conditions within one muscle type ($P>0.05$, t -test, Fig. 5). Furthermore, acetazolamide did not affect the disappearance of lactate from either muscle type during recovery. Muscle glycogen stores were significantly depleted immediately post-exercise ($P<0.05$, t -test), and this depletion was greater than could be accounted for by the build-up of lactate within the muscle alone (Fig. 6). Red muscle had significantly higher glycogen concentrations than white muscle at rest and throughout post-exercise recovery, and acetazolamide did not alter the pattern of glycogen depletion or restoration in either muscle type throughout the time course of the experiment (Fig. 6). Glucose concentrations did not change with regard to muscle type or treatment with acetazolamide (data not shown).

For comparison, lactate, glucose and glycogen concentrations in gill and hepatopancreas were also measured (Fig. 7). Both tissues had about the same resting

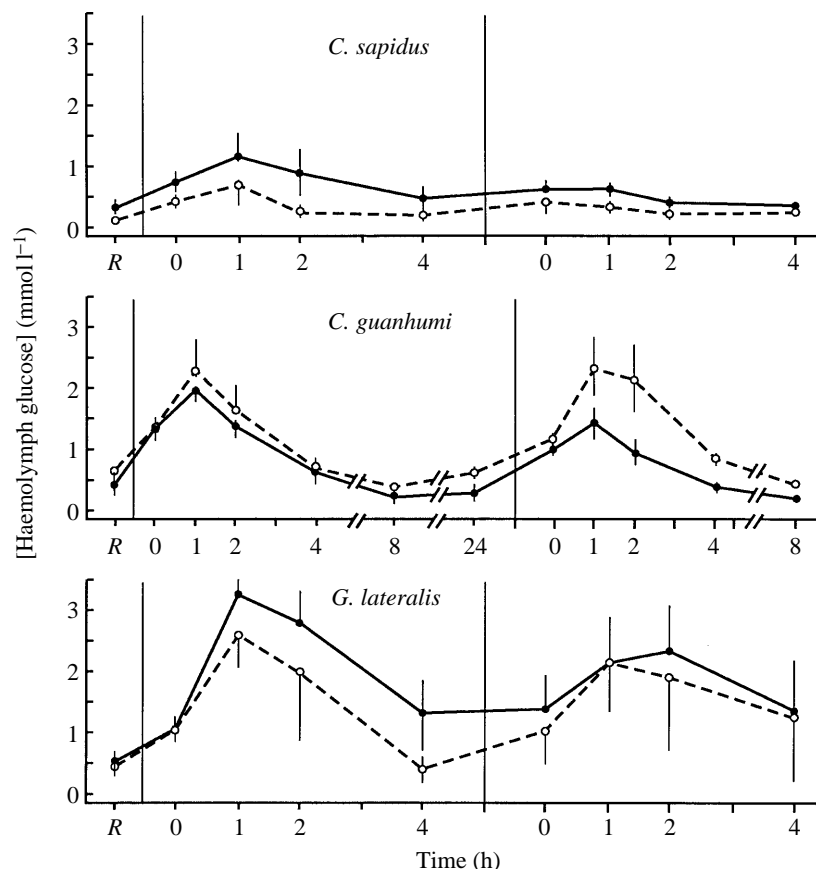


Fig. 4. Haemolymph glucose concentrations at rest (*R*) and during recovery after two successive bouts of exhaustive exercise in three species of decapod crustaceans. Dashed and solid lines and vertical bars as in Fig. 2. Mean \pm S.E.M. ($N=5$). $t=25^{\circ}\text{C}$.

glycogen concentration as white muscle, but the changes after exercise were much less pronounced. Lactate concentrations increased immediately after exercise but were restored to resting values by 4 h into recovery, whereas glucose concentrations did not change significantly. Acetazolamide did not alter the concentrations of any of the three metabolites in either tissue at rest or during post-exercise recovery (data not shown).

Fate of [¹⁴C]lactate

Injection of radiolabelled lactate resulted in detectable amounts of tracer in muscle tissue of all three species. The appearance of ¹⁴C in the glycogen fraction of muscle allowed for the calculation of a glycogen restoration rate (Table 3). The general trend was for *C. guanhumi* to have the highest rate, followed by *G. lateralis* and *C. sapidus*, but these differences were not significantly different because of high inter-individual variability. Radioactivity was also detected in other metabolic products (Table 4). Small percentages of the original label were detected in water for *C. sapidus* and in air for

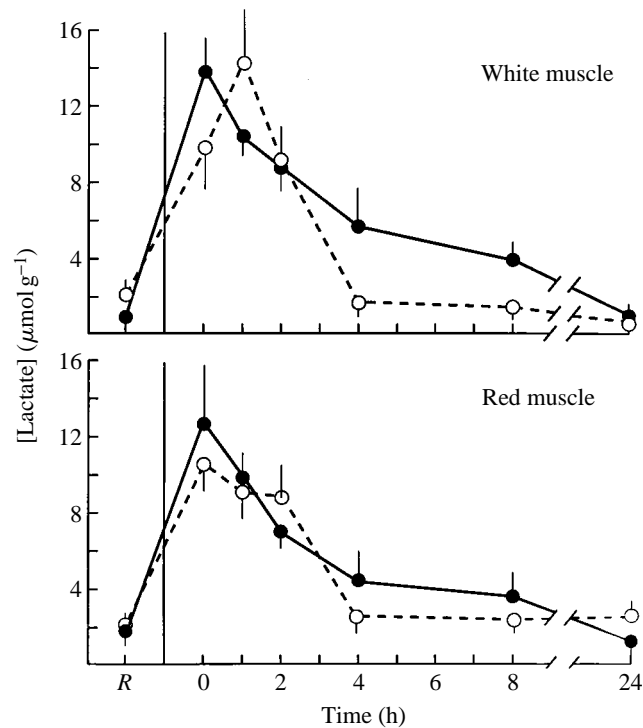


Fig. 5. Lactate concentrations in backfin (dorsal levator) muscle of *Callinectes sapidus* at rest (R) and during post-exercise recovery. Filled circles and solid lines represent saline-injected controls and open circles and dashed lines represent acetazolamide-treated (1 mmol l^{-1}) crabs. Mean \pm S.E.M. ($N=5-6$). The solid vertical line represents a 20 min exercise period.

Table 3. Glycogen restoration rates during post-exercise recovery in three species of decapod crustaceans

Species	Glycogen restoration rate ($\mu\text{mol glucosyl units g}^{-1} \text{ h}^{-1}$)
<i>Callinectes sapidus</i>	
Red levator	0.185 \pm 0.138
White levator	0.156 \pm 0.109
<i>Cardisoma guanhumii</i>	
Leg	0.476 \pm 0.307
<i>Gecarcinus lateralis</i>	
Leg	0.260 \pm 0.165

Mean \pm S.E.M. ($N=10$), $t=25^\circ\text{C}$.

C. guanhumii and *G. lateralis*, indicating that a portion of the lactate had been used as an energy source and metabolized to CO_2 . The total amount of radioactivity that was recovered as glycogen or as CO_2 or that remained as haemolymph lactate was small,

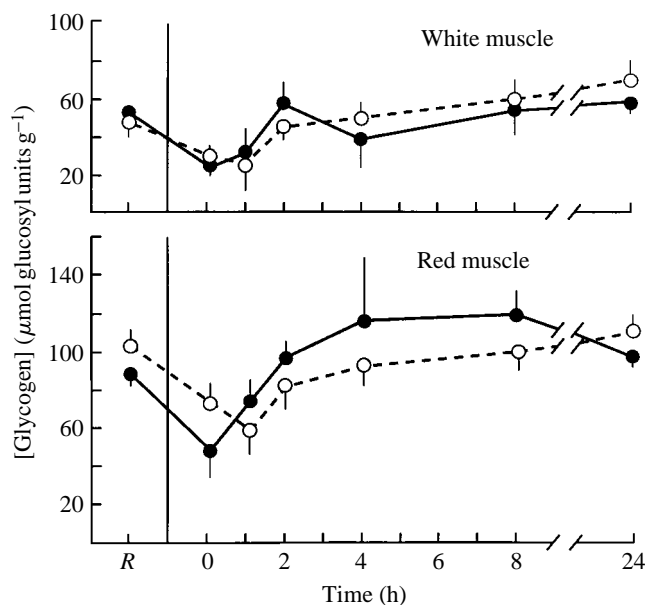


Fig. 6. Glycogen concentrations in backfin (dorsal levator) muscle of *Callinectes sapidus* at rest (R) and during post-exercise recovery. Filled and open circles and solid vertical line as in Fig. 5. Mean \pm S.E.M. ($N=5-6$).

Table 4. Distribution of ^{14}C from uniformly labelled lactate after 1 h of metabolism during the recovery period of crabs that had been exercised to exhaustion

Species	Percentage as CO_2	Percentage as muscle glycogen	Percentage as haemolymph lactate
<i>Callinectes sapidus</i>	2.87 \pm 0.40		9.87 \pm 0.77
Red levator		0.38 \pm 0.27	
White levator		0.47 \pm 0.34	
<i>Cardisoma guanhumi</i>	0.15 \pm 0.07		17.64 \pm 3.35
Leg		1.05 \pm 0.68	
<i>Gecarcinus lateralis</i>	0.23 \pm 0.10		15.20 \pm 1.54
Leg		0.56 \pm 0.36	

Mean \pm S.E.M. ($N=6$).

accounting for less than 20% of the original amount of injected label in each species (Table 4).

The [^{14}C]lactate washout curves from the haemolymph in all species were smooth and could be fitted to negative exponential curves with r^2 values greater than 0.85 (Fig. 8). Haemolymph total lactate concentrations remained fairly constant for *G. lateralis* only, allowing for the calculation of a lactate turnover rate for this species alone. This value was $2.149 \pm 1.225 \mu\text{mol lactate min}^{-1} \text{kg}^{-1}$ ($N=6$), and it is similar to the lactate turnover

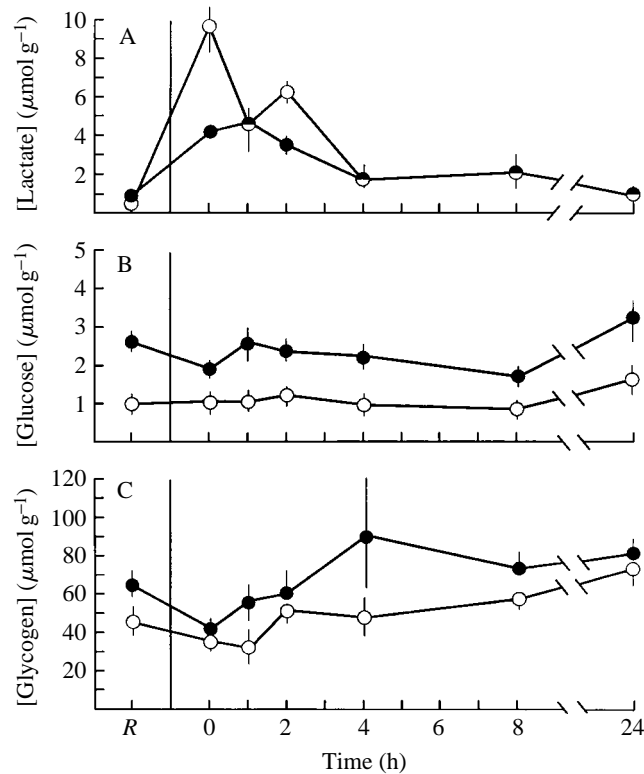


Fig. 7. Lactate (A), glucose (B) and glycogen (C) concentrations in gill (open circles) and hepatopancreas (filled circles) of *Callinectes sapidus* at rest (R) and during post-exercise recovery. Mean \pm S.E.M. ($N=5$). Solid vertical line as in Fig. 5.

value reported for salmonid fish (Milligan and McDonald, 1988). In contrast to earlier experiments, in which fewer haemolymph samples were taken (e.g. Fig. 3), *C. sapidus* and *C. guanhumi* haemolymph lactate concentrations increased over the duration of the sampling period (Fig. 8 insets), violating one of the major assumptions of the models used to calculate turnover rates. It is likely that these increases were due to the protocol used for multiple sampling, which may have interfered with the crabs' abilities to recover from anaerobic metabolism (discussed below).

Discussion

Comparative exercise performance

From our observations, it appears that *C. sapidus* is the most suited of the three species for sustained high levels of activity. It easily maintains 20 min of vigorous exercise and it also can withstand up to 1 h of vigorous swimming (Booth *et al.* 1982). It utilizes both aerobic and anaerobic pathways of energy production; oxygen uptake increases 2.6-fold and lactate accumulates in both the haemolymph and muscle (Booth *et al.* 1982, 1984; Figs 1, 2, Table 2). In contrast, both terrestrial species fatigue quickly, appearing to

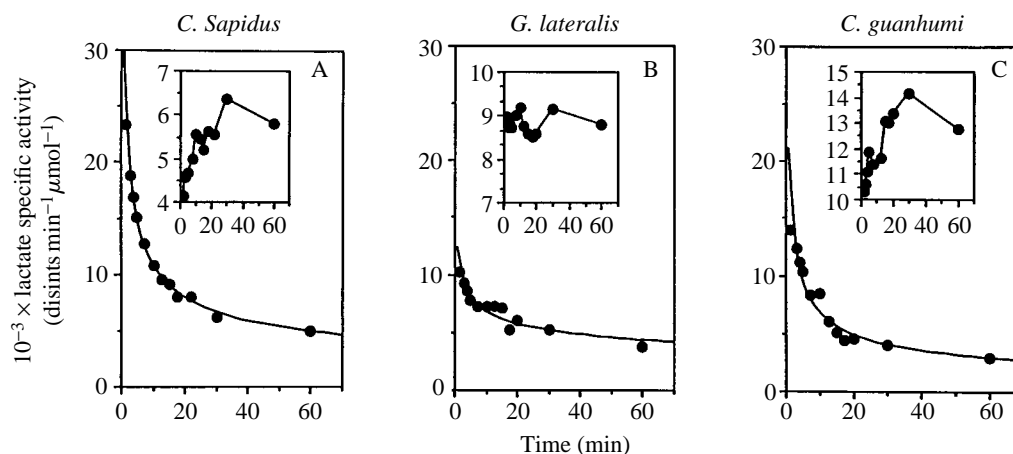


Fig. 8. Representative examples of haemolymph lactate specific activity *versus* time post-injection in *Callinectes sapidus* (A), *Gecarcinus lateralis* (B) and *Cardisoma guanhumi* (C). Insets show total haemolymph lactate concentrations (mmol l^{-1}) in the same samples. The period examined was 1–2 h post-exercise.

depend more on anaerobic energy production. *C. guanhumi* accumulates haemolymph lactate at the fastest rate (Fig. 1) and it has the highest lactate concentrations in both haemolymph and muscle (Fig. 2, Table 2). The ability of this species to maintain a short but vigorous state of exercise anaerobically may be a result of the relatively high concentrations of glycogen in its locomotor muscles and of the high glycogen restoration rate compared with the other species (Tables 2, 3). These comparisons are supported by results on the relative capabilities of aerobic exercise in terrestrial crabs. *G. lateralis* displays the lowest running speed (0.16 km h^{-1}) at which fatigue sets in; the value for *C. guanhumi* is similar at 0.18 km h^{-1} (Herreid *et al.* 1979, 1983). These values are significantly lower than the velocity required to induce fatigue (0.30 km h^{-1}) in a highly aerobic terrestrial crab *Ocypode gaudichaudii* (Full and Herreid, 1983). It appears that *C. sapidus*, *C. guanhumi* and *G. lateralis* possess both differential capacities for exercise and differential reliance on aerobic and anaerobic energy production.

Gluconeogenesis and the metabolism of lactate

The incorporation of [^{14}C]lactate into glycogen in muscle indicates the presence of gluconeogenic capability in all three species, but the rate of gluconeogenesis is slow, and the amount of radioactivity recovered in glycogen is at most only about 1% of the original injected label (Tables 3 and 4). Furthermore, a significant amount of the label remains in the haemolymph as unmetabolized lactate. In addition, a small fraction of the total radioactivity was detectable as CO_2 gas in the medium, indicating that some of the lactate has been metabolised as an energy source. Although our results probably underestimate CO_2 production rates, because they did not take into account how much of the label remained as haemolymph $\text{H}^{14}\text{CO}_3^-$, they nevertheless strongly suggest that there are multiple metabolic fates for lactate. Other crustaceans appear to show the same

pattern, with lactate being metabolized to glucose, amino acids and CO₂ (Thabrew *et al.* 1971; Gade *et al.* 1986; van Aardt, 1988). It is possible that the bulk of the unrecovered label was distributed among intracellular metabolite pools, including the amino acid pool, and as lipids and bicarbonate.

The potential pathway of crustacean gluconeogenesis is also intriguing. Crustaceans have been documented to have significant levels of activity for enzymes of glycolysis, but gluconeogenic enzyme activities have been either very low or absent, indicating a gluconeogenic pathway other than the Cori cycle (Thabrew *et al.* 1971; Walsh and Henry, 1990; Lallier and Walsh, 1991a). Crustacean metabolic potential appears to be geared towards the metabolism of amino acids rather than carbohydrates (Walsh and Henry, 1990).

Similar low activities of gluconeogenic enzymes have been found in white muscle of fishes (Suarez and Mommsen, 1987), yet current physiological evidence strongly suggests that the muscle is a major site for post-exercise gluconeogenesis from lactate (Milligan and McDonald, 1988; Walsh, 1989). Recent studies on fish, which have shown that energy charge recovers much more quickly than does lactate/glycogen metabolism, suggest that gluconeogenesis occurs *via* the reversal of pyruvate kinase, a pathway that does not require bicarbonate (Schulte *et al.* 1992). A similar situation could be occurring in crustaceans. Gluconeogenesis is important in maintaining a constant circulatory supply of metabolic fuel (glucose) to the central nervous system (CNS) in higher vertebrates after exercise (reviewed by Hers and Hue, 1983). It is possible that in crustaceans, which lack a well-developed CNS, there was no selective advantage in the efficient recycling of lactate to circulating glucose. That fact, coupled with the relatively low metabolic rates shown by both crustaceans and fish, could have delayed the evolutionary development of the Cori cycle until the emergence of the combination of a highly developed CNS and the higher metabolic rates found in the terrestrial vertebrates.

The values for lactate clearance from the haemolymph for *G. lateralis* (Fig. 8) are similar to those reported for fish (Milligan and McDonald, 1988). Interestingly, of the three species used in the study, only *G. lateralis* met the requirement for constant total haemolymph lactate concentration during the sampling period. In both *C. sapidus* and *C. guanhumi*, lactate concentrations increased. Crustaceans must be handled at each sampling time, because indwelling catheters, which immediately become clogged with clotted haemolymph, are not an option as they are in fish. These physiological differences dictated a difference in experimental protocol, which resulted in strict limitations for the work on crabs. *G. lateralis*, the least active (and least sensitive to handling stress), was the only reliable animal model for this approach.

Gluconeogenesis and the role of carbonic anhydrase

Regardless of the pathway of gluconeogenesis, or the specific tissues believed to be involved, it appears that in crustaceans carbonic anhydrase is not a critical enzyme for supplying HCO₃⁻ for pyruvate carboxylation. Acetazolamide has no effect on haemolymph lactate build-up in animals given repeated bouts of exercise and it has no effect on muscle lactate or glycogen dynamics (Figs 3 and 5). Furthermore, acetazolamide did not abolish the pattern of haemolymph hyperglycaemia during post-

exercise recovery (Fig. 4). This is in contrast to results from lower vertebrates given an almost identical experimental treatment (Herbert *et al.* 1983). In those studies, CA inhibition by acetazolamide caused a decreased blood lactate concentration compared with controls and eliminated blood hyperglycaemia after the second exercise period. Those results were interpreted as evidence for a role of CA in gluconeogenesis (i.e. CA inhibition caused bicarbonate availability to be limiting for the carboxylation of pyruvate, thus retarding glycogen restoration and causing the lower blood lactate levels after the second exercise bout) (Herbert *et al.* 1983). Data presented in this study indicate no such involvement of CA in lactate metabolism.

Gluconeogenesis in crustacean tissue appears to proceed either by a pathway that is independent of pyruvate carboxylation or *via* the Cori cycle, but at such a low rate that the rapid catalysis of CO_2 to HCO_3^- is not necessary to facilitate the pathway. Support for these conclusions also comes from gluconeogenic studies on the gills of *Carcinus maenas* (Thabrew *et al.* 1971). There was no difference in the rate of gluconeogenesis in gill tissue incubated with $[^{14}\text{C}]$ lactate in the absence or in the presence of bicarbonate, strongly suggesting that bicarbonate availability is not a critical factor in the process.

Carbonic anhydrase distribution and function

In the light of the physiological evidence, which seems to discount the role of CA in gluconeogenesis, the distribution of CA activity does not appear to be related to intermediary metabolism. CA levels in the gill can be correlated with environmental salinity and the enzyme's role in ion regulation (Henry and Cameron, 1982, 1983; Henry, 1984) and with the high concentration of mitochondria-rich chloride cells in the posterior ion-transporting lamellae (Neufeld *et al.* 1980).

If CA is not involved in muscle gluconeogenesis, it may have a respiratory function. The limiting step in CO_2 transport, the catalysed conversion of HCO_3^- to CO_2 , occurs at the boundary layer of the membrane (Gutknecht *et al.* 1977). Thus, cytoplasmic CA may be necessary to keep intracellular P_{CO_2} high enough to drive diffusion into the plasma, as postulated for mammalian muscle by Gros and Dodgson (1988).

The only tissue that shows anomalously high CA activity is the hepatopancreas (both cytoplasm and mitochondria) in *G. lateralis*. This suggests a metabolic function for CA in this tissue that is not operating in the other two species. The fully terrestrial nature of this species also suggests that the putative metabolic function of hepatopancreatic CA would be in nitrogen metabolism (e.g. urea and/or uric acid synthesis). Terrestrial crabs are known to produce both compounds, while semi-terrestrial species of the genus *Cardisoma* have been shown to be primarily ammoniotelic (Horne, 1968; Henry and Cameron, 1981; Wood *et al.* 1986, Greenaway and Morris, 1989). Furthermore, the hepatopancreas is believed to be the sole site of purine metabolism in crustaceans (Greenaway and Morris, 1989; Dykens, 1991; Lallier and Walsh, 1991*b*). CA in hepatocytes of fish has been shown to be vital to urea synthesis (Walsh *et al.* 1989), and it is plausible for the enzyme to have an analogous role in crustacean metabolism. This avenue of research also deserves further investigation.

The authors would like to thank Jeff Shimonski of Parrot Jungle and Gardens, Miami,

Florida, for collecting the *Cardisoma*. The work was supported by NSF DCB 88-01926 and IBN 93-04844 to R.P.H. and NSF DCB 86-08728 and IBN 91-18819 to P.J.W. C.E.B. was supported by a Connecticut State University Faculty Research Grant. Partial support was also received from the Alabama Agricultural Experiment Station.

References

- BALLANTYNE, J. S. AND STOREY, K. B. (1984). Mitochondria from the hepatopancreas of the marine clam *Mercenaria mercenaria*: substrate preferences and salt and pH effects on the oxidation of palmitoyl-L-carnitine and succinate. *J. exp. Zool.* **230**, 165–174.
- BOOTH, C. E., MCMAHON, B. R., DEFUR, P. L. AND WILKES, P. R. H. (1984). Acid–base regulation during exercise and recovery in the blue crab, *Callinectes sapidus*. *Respir. Physiol.* **58**, 359–376.
- BOOTH, C. E., MCMAHON, B. R. AND PINDER, A. W. (1982). Oxygen uptake and the potentiating effects of increased hemolymph lactate on oxygen transport during exercise in the blue crab, *Callinectes sapidus*. *J. comp. Physiol.* **148B**, 111–121.
- DODGSON, S. J. AND FORSTER, R. E. (1986). Inhibition of CA V decreases glucose synthesis from pyruvate. *Archs Biochem. Biophys.* **251**, 198–204.
- DYKENS, J. A. (1991). Purinolytic capacity and origin of hemolymph urate in *Carcinus maenas* during hypoxia. *Comp. Biochem. Physiol.* **98B**, 579–586.
- FORSTER, M. E., WALDRON, F. M. AND TAYLOR, H. H. (1989). Recovery from exhausting exercise in a bimodally breathing crab, *Leptograpsus variegatus* (Decapoda: Grapsidae). *J. exp. mar. Biol. Ecol.* **127**, 165–173.
- FULL, R. J. AND HERREID, C. F. (1983). Aerobic response to exercise of the fastest land crab. *Am. J. Physiol.* **244**, R530–R536.
- GADE, G., GRAHAM, R. A. AND ELLINGTON, W. R. (1986). Metabolic disposition of lactate in the horseshoe crab *Limulus polyphemus* and the stone crab *Menippe mercenaria*. *Mar. Biol.* **91**, 473–479.
- GLEESON, R. A. AND ZUBKOFF, P. L. (1977). The determination of hemolymph volume in the blue crab, *Callinectes sapidus*, using ¹⁴C-thiocyanate. *Comp. Biochem. Physiol.* **56A**, 411–414.
- GLEESON, T. T. AND DALESSIO, P. M. (1989). Lactate and glycogen metabolism in the lizard *Dipsosaurus dorsalis* following exhaustive exercise. *J. exp. Biol.* **144**, 377–393.
- GLEESON, T. T. AND DALESSIO, P. M. (1990). Lactate: a substrate for reptilian muscle gluconeogenesis following exhaustive exercise. *J. comp. Physiol.* **160B**, 331–338.
- GREENAWAY, P. AND MORRIS, S. (1989). Adaptations to a terrestrial existence by the robber crab, *Birgus latro* L. III. Nitrogenous excretion. *J. exp. Biol.* **143**, 333–346.
- GROS, G. AND DODGSON, S. J. (1988). Velocity of CO₂ exchange in muscle and liver. *A. Rev. Physiol.* **50**, 669–694.
- GUTKNECHT, J., BISSON, M. A. AND TOSTESON, F. C. (1977). Diffusion of carbon dioxide through lipid bilayer membranes. Effects of carbonic anhydrase, bicarbonate and unstirred layers. *J. gen. Physiol.* **69**, 779–794.
- HENRY, R. P. (1984). The role of carbonic anhydrase in blood ion and acid–base regulation. *Am. Zool.* **24**, 241–251.
- HENRY, R. P. (1987). Quaternary ammonium sulfanilamide: a membrane-impermeant carbonic anhydrase inhibitor. *Am. J. Physiol.* **252**, R959–R965.
- HENRY, R. P. (1991). Techniques for measuring carbonic anhydrase activity *in vitro*: the electrometric delta pH and pH stat methods. In *The Carbonic Anhydrases: Cellular Physiology and Molecular Genetics* (ed. S. J. Dodgson, R. E. Tashian and G. Gros), pp. 119–124. New York: Plenum Press.
- HENRY, R. P. AND CAMERON, J. N. (1981). A survey of blood and tissue nitrogen compounds in terrestrial decapods in Palau. *J. exp. Zool.* **218**, 83–88.
- HENRY, R. P. AND CAMERON, J. N. (1982). The distribution and partial characterization of carbonic anhydrase in selected aquatic and terrestrial decapod crustaceans. *J. exp. Zool.* **221**, 309–321.
- HENRY, R. P. AND CAMERON, J. N. (1983). The role of carbonic anhydrase in respiration, ion regulation and acid–base balance in the aquatic crab, *Callinectes sapidus*, and the terrestrial crab, *Gecarcinus lateralis*. *J. exp. Biol.* **103**, 205–223.
- HENRY, R. P. AND KORMANIK, G. A. (1985). Carbonic anhydrase activity and calcium deposition during the molt cycle of the blue crab, *Callinectes sapidus*. *J. Crust. Biol.* **5**, 234–241.

- HERBERT, J. D., COULSON, R. A. AND HERNANDEZ, T. (1983). Inhibition of pyruvate carboxylation in alligators and chameleons by carbonic anhydrase inhibitors. *Comp. Biochem. Physiol.* **75A**, 185–192.
- HERREID, C. F., LEE, L. AND SHAH, G. M. (1979). Respiration and heart rate in exercising land crabs. *Respir. Physiol.* **36**, 109–120.
- HERREID, C. F., O'MAHONY, P. M. AND FULL, R. J. (1983). Locomotion in land crabs: respiratory and cardiac responses of *Gecarcinus lateralis*. *Comp. Biochem. Physiol.* **74A**, 117–124.
- HERS, H. G. AND HUE, L. (1983). Gluconeogenesis and related aspects of glycolysis. *A. Rev. Biochem.* **52**, 617–653.
- HORNE, F. R. (1968). Nitrogen excretion in Crustacea. I. The herbivorous land crab *Cardisoma guanhumi* Latreille. *Comp. Biochem. Physiol.* **26**, 687–695.
- JOHNSON, T. A. AND FUSARO, R. M. (1966). The quantitative enzymic determination of animal liver glycogen. *Analyt. Biochem.* **15**, 140–149.
- KEPPLER, D. AND DECKER, K. (1984). Glycogen determination with amyloglucosidase. In *Methods of Enzymatic Analysis*, 2nd edn, vol. 3 (ed. H. U. Bergmeyer), pp. 1127–1131. Weinheim: VCH Verlagsgesellschaft.
- LALLIER, F. H. AND WALSH, P. J. (1991a). Metabolic potential in tissues of the blue crab, *Callinectes sapidus*. *Bull. mar. Sci.* **48**, 665–669.
- LALLIER, F. H. AND WALSH, P. J. (1991b). Activities of uricase, xanthine oxidase and xanthine dehydrogenase in the hepatopancreas of aquatic and terrestrial crabs. *J. Crust. Biol.* **11**, 506–512.
- LALLIER, F. H. AND WALSH, P. J. (1992). Metabolism of isolated hepatopancreas cells from the blue crab (*Callinectes sapidus*) under simulated postexercise and hypoxic conditions. *Physiol. Zool.* **65**, 712–723.
- MAREN, T. H. (1967). Carbonic anhydrase in the animal kingdom. *Fedn Proc. Fedn Am. Socs exp. Biol.* **26**, 1097–1103.
- MCDONALD, D. G., MCMAHON, B. R. AND WOOD, C. M. (1979). An analysis of acid–base disturbances in the haemolymph following strenuous activity in the dungeness crab, *Cancer magister*. *J. exp. Biol.* **79**, 47–58.
- MILLIGAN, C. L. AND MCDONALD, D. G. (1988). *In vivo* lactate kinetics at rest and during recovery from exhaustive exercise in coho salmon (*Oncorhynchus kisutch*) and starry flounder (*Platichthys stellatus*). *J. exp. Biol.* **135**, 119–131.
- MOMMSEN, T. P., FRENCH, C. J. AND HOCHACHKA, P. W. (1980). Sites and patterns of protein and amino acid utilization during spawning migration of salmon. *Can. J. Zool.* **58**, 1785–1799.
- MORRIS, S. AND GREENAWAY, P. (1989). Adaptations to a terrestrial existence in the robber crab, *Birgus latro* L. IV. L-lactate dehydrogenase function and L-lactate accumulation during exercise. *Comp. Biochem. Physiol.* **94B**, 59–64.
- NEUFELD, G. J., HOLIDAY, C. W. AND PRITCHARD, J. B. (1980). Salinity adaptation of gill Na,K-ATPase in the blue crab, *Callinectes sapidus*. *J. exp. Zool.* **211**, 215–224.
- ROCA, P., GIANOTTI, M. AND PALOU, A. (1985). Enzymatic lactate-specific radioactivity determination in biological samples. *Analyt. Biochem.* **148**, 190–193.
- SCHULTE, P. M., MOYES, C. D. AND HOCHACHKA, P. W. (1992). Integrating metabolic pathways in post-exercise recovery of white muscle. *J. exp. Biol.* **166**, 181–195.
- SMATRESK, N. J., PRESLAR, A. J. AND CAMERON, J. N. (1979). Post-exercise acid–base disturbance in *Gecarcinus lateralis*, a terrestrial crab. *J. exp. Zool.* **210**, 205–210.
- SUAREZ, R. K. AND MOMMSEN, T. P. (1987). Gluconeogenesis in teleost fish. *Can. J. Zool.* **65**, 1869–1882.
- THABREW, M. I., POAT, P. C. AND MUNDAY, K. A. (1971). Carbohydrate metabolism in *Carcinus maenas* gill tissue. *Comp. Biochem. Physiol.* **40B**, 531–541.
- VAN AARDT, W. J. (1988). Lactate metabolism and glucose patterns in the river crab, *Potamonautes warreni* Calman, during anoxia and subsequent recovery. *Comp. Biochem. Physiol.* **91A**, 299–304.
- WALSH, P. J. (1989). An *in vitro* model of post-exercise hepatic gluconeogenesis in the gulf toadfish, *Opsanus beta*. *J. exp. Biol.* **147**, 393–406.
- WALSH, P. J. AND HENRY, R. P. (1990). Activities of metabolic enzymes in the deep-water crabs *Chaceon fenneri* and *C. quinqueedens* and the shallow-water crab *Callinectes sapidus*. *Mar. Biol.* **106**, 343–346.
- WALSH, P. J., PARENT, J. J. AND HENRY, R. P. (1989). Carbonic anhydrase supplies bicarbonate for urea synthesis in toadfish (*Opsanus beta*) hepatocytes. *Physiol. Zool.* **62**, 1257–1272.

- WOOD, C. M., BOUTILIER, R. G. AND RANDALL, D. J. (1986). The physiology of dehydration stress in the land crab, *Cardisoma carnifex*: respiration, ionoregulation, acid–base balance and nitrogenous waste excretion. *J. exp. Biol.* **126**, 271–296.
- WOOD, C. M. AND RANDALL, D. J. (1981). Haemolymph gas transport, acid–base regulation and anaerobic metabolism during exercise in the land crab (*Cardisoma carnifex*). *J. exp. Zool.* **218**, 23–35.