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HAEMOLYMPH ACID–BASE, ELECTROLYTE AND GAS STATUS DURING SUSTAINED VOLUNTARY ACTIVITY IN THE LAND HERMIT CRAB (*COENOBITA COMPRESSUS*)

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SUMMARY

After 3 h (50 m) of voluntary walking, the haemolymph pH of the land hermit crab *Coenobita compressus* (H. Milne Edwards) decreased by 0·4 units. This was accompanied by increases in CO_2 tension (P_{CO_2}), bicarbonate ($HCO_3^- + CO_3^{2-}$) and lactate concentrations. The hypercapnic acidosis was partially compensated by metabolic bicarbonate accumulation and an H^+ deficit developed. Unloaded crabs accumulated less of a proton load than crabs transporting mollusc shells. During activity, oxygenation of the haemocyanin (HCy) accounted for the release of 0·3 mmol CO_2 l^{-1}, *via* the Haldane effect, which was seven times more than in settled crabs. Control acid–base balance was re-established within 1 h of recovery. At this time, acidic equivalents were excreted at a mean flux rate of 5 mequiv kg^{-1} h^{-1} into a source of external water. $[Na^+]$ and the ratio of $[Na^+]:[Cl^-]$ increased during exercise.

Coenobita haemolymph had a high O_2-carrying capacity ($C^{max}_{HCyO_2} = 1{\cdot}55$ mmol l^{-1}). HCy oxygen-binding characteristics were typical of other decapods ($\phi = -0{\cdot}44$), yet no lactate sensitivity was apparent. Settled *in vivo* values of O_2 tension (P_{O_2}) and content (C_{O_2}) were located around the half-saturation tension (P_{50}) of the dissociation curve. During exercise, P_{O_2} increased and an unopposed Bohr shift decreased the O_2-binding affinity, thereby reducing postbranchial saturation. Quantitatively, however, compensations in cardiac output ($\dot{V}b$) were more instrumental in increasing the O_2 delivery to respiring tissues. During recovery, haemolymph P_{O_2} remained high and the venous reserve doubled.

INTRODUCTION

The effect of a short burst of exhausting activity on haemolymph acid–base balance and gas transport has been studied in decapod crustaceans (McMahon,

Key words: voluntary exercise, hermit crab, *Coenobita compressus*, acid–base status during exercise, electrolyte status during exercise, blood gas status during exercise.

McDonald & Wood, 1979; Smatresk, Preslar & Cameron, 1979; Wood & Randall, 1981*a*,*b*). The accompanying acidosis is frequently of mixed respiratory and metabolic origin requiring several hours for recovery. Sustained submaximal exercise (swimming for 1 h) has been studied in the blue crab *Callinectes sapidus* (Booth, McMahon, de Fur & Wilkes, 1984). These authors reported a similar acid–base response [i.e. increases in CO_2 tension (P_{CO_2}) and lactate, decrease in total CO_2]. This was most pronounced after 15 min but recovered partially within 1 h, despite the continued accumulation of lactate because of the transfer of acidic equivalents to the ambient sea water *via* branchial electroneutral ion exchanges. This continued during recovery to restore pH fully.

Mechanisms employed to maintain O_2 transport and delivery also differ between short-burst and sustained submaximal exercise. During short periods of enforced pedestrian activity, O_2 uptake ($\dot{M}_{O_2}$) increases through compensations in ventilation ($\dot{V}w$), gill and tissue perfusion ($\dot{V}b$), mean pressure gradient for diffusion (ΔP_g) and O_2 conductance (ΔTO_2 – see review articles by Herreid, 1981; McMahon, 1981; McMahon & Wilkens, 1983). The respiratory pigment haemocyanin (HCy) becomes increasingly involved in O_2 delivery. Postbranchial P_{O_2} decreases from saturated values onto the descending portion of the dissociation curve, releasing O_2 from the venous reserve. Nonetheless, a shortfall in O_2 delivery results in a shift towards anaerobic metabolism. It was originally thought that the Bohr shift was the major effector of HCy O_2-binding affinity during exercise. By contrast, sustained swimming in blue crabs is fuelled predominantly aerobically and internal P_{O_2} remains high (Booth, McMahon & Pinder, 1982). Furthermore, O_2 delivery is increased through cardiovascular adaptations rather than a change in the role of the pigment since the Bohr effect is minimized by a lactate-induced increase in O_2-binding affinity.

We recently discovered the capability for sustained submaximal walking (up to 3 h) in a tropical land hermit crab *Coenobita compressus* (H. Milne Edwards) and developed a rotating respirometer to characterize respiratory gas exchange (Wheatly, McMahon, Burggren & Pinder, 1985). In the present study we examined haemolymph acid–base and electrolyte status and gas transport during voluntary activity. Hermit crabs transport a substantial water reservoir inside the molluscan shell which they will replenish from an external source (Wheatly, Burggren & McMahon, 1984). By sampling this 'replacement water' we were able to determine acidic equivalent exchange, known to be an effective mechanism of acid–base regulation in aquatic species (see reviews by Heisler, 1984; Cameron, 1986).

MATERIALS AND METHODS

Protocol

Land hermit crabs (*Coenobita compressus* $10{\cdot}5 \pm 1{\cdot}1$ g, $N = 29$) were collected and maintained in Panama as described previously (Wheatly *et al.* 1985). Post- (designated a) and prebranchial (v) haemolymph were sampled for pH, O_2 and CO_2 tension (P_{O_2} and P_{CO_2}) and concentrations of lactate and inorganic ions at rest, after 50 m (150 min at $0{\cdot}6\,cm\,s^{-1}$) of voluntary walking in a rotating respirometer or

following 1 h of recovery. After removing crabs from their shells, postbranchial haemolymph was sampled from the pericardium *via* a hole made in the carapace which had previously been sealed with a latex septum, and prebranchial haemolymph was sampled from the infrabranchial sinus at the base of a walking leg (see McMahon & Burggren, 1979). Only one sample was removed from each animal, constituting an unpaired experimental design. Acid–base exchange with external sea water was assessed in inactive crabs and following voluntary exercise after the introduction of 20 ml of 10 % sea water into the bottom of the respirometer. In a parallel series of experiments, crabs were removed from their shells to assess the effects of loading. To assist in interpreting blood gas data, haemolymph CO_2- and O_2-binding characteristics were assessed *in vitro* on haemolymph removed from 60 crabs which had been transported back to Calgary.

Analytical procedures

Haemolymph

Haemolymph (300 μl) was analysed immediately for pH, P_{O_2} and P_{CO_2} using Instrumentation Laboratory electrodes (20982–pH, 20984–O_2 and 20983–CO_2) thermostatted to 30 ± 2°C and connected to an IL 213 blood gas analyser. The pH electrode was calibrated with Radiometer precision buffers (S1500 and S1510). The CO_2 electrode was calibrated with 3 % CO_2 in N_2 to an arbitrary value of 70 using scale expansion. The calibration gas was then serially diluted twice with air (1 % and 0·3 % CO_2 approximately) and the meter reading regressed against P_{CO_2}. The precise composition of these calibration gases was calculated from measured P_{O_2}.

Prebranchial haemolymph (80 μl) was deproteinized in 400 μl of ice-cold perchloric (10 % w/v) and analysed within 2 weeks for [lactate] using a Sigma diagnostic kit (826 UV). In some species, Cu^{2+} liberated from HCy can artificially elevate values unless a modified procedure is adopted (see Graham, Mangum, Terwilliger & Terwilliger, 1983). Remaining haemolymph was frozen and subsequently analysed in Calgary for major inorganic ions using methods outlined in Wheatly *et al.* (1984).

Water

The net flux of acidic (i.e. H^+ or NH_4^+) or basic (OH^- or HCO_3^-) equivalents between the crab and replaced sea water was determined over a 1-h period of rest or recovery from exercise using procedures outlined by McDonald & Wood (1981). Titratable alkalinity [$TA-HCO_3^-$] was determined by titrating 5 ml of water with 0·02 mol l^{-1} HCl to an end pH of 4·0 as determined on a Radiometer GK 240 2C combination electrode coupled to a PHM 84 pH meter. Theoretically this technique titrates any buffers with pK values between 3 and 9, which includes organic bases (see Heisler, 1984). These, however, are produced primarily at the antennal gland. It is unlikely that the water replaced in the present study comes into contact with the third antennal segment and so there is less possibility of contamination than in an uncatheterized aquatic species. Ammonia [$NH_3+NH_4^+$] was determined by the phenolhypochlorite method (Solorzáno, 1969). Changes in [$TA-HCO_3^-$] and [$NH_3+NH_4^+$] were converted to fluxes and combined (signs considered) to give net

acidic equivalent flux. This cannot distinguish between net acid excretion and base uptake.

In vitro *analysis*

Haemolymph O_2 and CO_2 equilibrium curves were constructed *in vitro* in Calgary and subsequently used to interpolate O_2 and CO_2 contents (C_{O_2} and C_{CO_2}, respectively) from *in vivo* measured tensions. CO_2 equilibrium curves and non-bicarbonate buffer curves were determined at 30°C on haemolymph pooled from eight animals using techniques outlined in Truchot (1976) and Randall & Wood (1981). Declotted haemolymph (2·0 ml) was equilibrated with humidified gases (P_{CO_2} = 1·3–46·7 Torr; P_{O_2} = 0–138 Torr) obtained *via* Wösthoff mixing pumps from analysed mixtures. After 45 min, pH (50-μl samples) and C_{CO_2} (40-μl samples) were measured with a Radiometer (G299A) liquid-junction capillary electrode connected to a BMS 3 MK 2 Blood Micro System and a Corning 965 CO_2 analyser, respectively.

A conventional gasometric technique was used to determine O_2-combining characteristics of pooled haemolymph (see Wheatly & McMahon, 1982). After equilibration with P_{O_2} up to 70 Torr, measurements were made of C_{O_2}, using a Lex-O_2 Con automatic analyser (Lexington Inst. – 80-μl samples), and P_{O_2}, using a Radiometer O_2 electrode and acid–base analyser (E5047; PHM 71/72 – 200-μl samples). CO_2 was used as an exogenous buffer (P_{CO_2} = 2·7, 13·3, 26·5 Torr) to study binding over the pH range 7·2–7·7. Haemolymph O_2-carrying capacity ($C_{O_2}^{max}$) was determined on an air-equilibrated sample. Lactate sensitivity was investigated using methodology outlined by Booth *et al.* (1982). A pooled haemolymph sample was divided into three aliquots and [lactate] adjusted to 3·3, 6·5 or 12·9 mmol l^{-1} using 0·2 mol l^{-1} neutral (Li^+) – lithium lactate (the effect of Li^+ was assumed to be negligible). Oxygen dissociation curves were constructed at equilibration P_{CO_2} of 2·7 or 26·5 Torr.

Statistical treatment of the data

All data are expressed as mean ± S.E. (number of observations). Samples were tested for homogeneity of variance (F-test) and means compared by Student's two-tailed *t*-test (unpaired variates) using $P = 0{\cdot}05$ as the confidence limit. Linear regression was performed by the least-squares method. Slopes from different treatments were compared using an analysis of covariance. In cases where regression lines were parallel, coincidence was tested using the Newman–Keuls multiple comparison procedure.

RESULTS

Acid–base balance

In vitro *haemolymph CO_2 combining properties*

CO_2-combining characteristics of oxygenated and deoxygenated haemolymph revealed a Haldane effect. Deoxygenated haemolymph contained more CO_2 at a

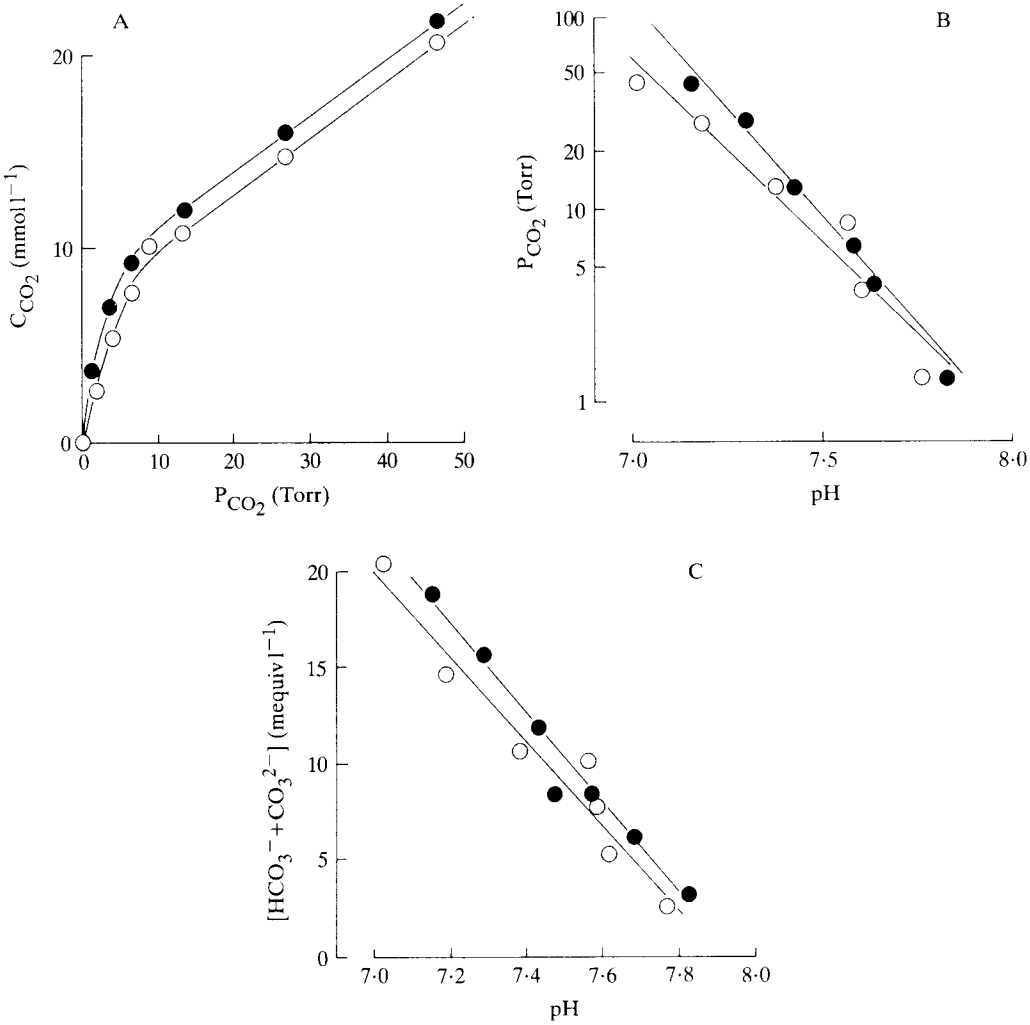

Fig. 1. (A) *In vitro* determined CO_2 dissociation curves for oxygenated (○) and deoxygenated (●) *Coenobita compressus* serum. (B) Log P_{CO_2} as a function of pH for oxygenated (○) and deoxygenated (●) serum. Regression equations: deoxygenated log $P_{CO_2} = 17{\cdot}9 - 2{\cdot}3$pH ($r = 0{\cdot}994$; $N = 7$); oxygenated log $P_{CO_2} = 14{\cdot}9 - 1{\cdot}9$pH ($r = 0{\cdot}961$; $N = 7$). (C) [$HCO_3^- + CO_3^{2-}$] as a function of pH in oxygenated (○) and deoxygenated (●) *C. compressus* serum. Regression equations: deoxygenated [$HCO_3^- + CO_3^{2-}$] = $189{\cdot}9 - 24{\cdot}0$pH ($r = 0{\cdot}983$; $N = 7$); oxygenated [$HCO_3^- + CO_3^{2-}$] = $176{\cdot}4 - 22{\cdot}3$pH ($r = 0{\cdot}975$; $N = 7$). Slope indicates non-bicarbonate buffering capacity (β) – mean value $-23{\cdot}1$ mmol l^{-1} pH unit^{-1}.

given P_{CO_2}, except at low P_{CO_2} values where the two lines converged (Fig. 1A). Correspondingly, the pH at constant P_{CO_2} was consistently higher in deoxygenated haemolymph (Fig. 1B). The slopes were not significantly different but the elevations were ($0{\cdot}01 > P > 0{\cdot}005$) suggesting that the lines were not coincident even though they did converge around pH 7·92.

Bicarbonate concentration (effectively $[HCO_3^- + CO_3^{2-}]$) was calculated from these data as $C_{CO_2} - \alpha CO_2 \times P_{CO_2}$ (using a CO_2 solubility coefficient, αCO_2, of $0{\cdot}0352\,mmol\,l^{-1}\,Torr^{-1}$) and plotted *versus* pH (Fig. 1C). The slopes of these non-bicarbonate buffer lines were not significantly different but the elevations were ($0{\cdot}01 > P > 0{\cdot}005$). The combined non-bicarbonate buffer value (β) was 23 mequiv l^{-1} pH $unit^{-1}$ (slykes) over the physiological pH range.

In vivo *haemolymph acid–base status*

Using *in vitro* CO_2-binding data, $[HCO_3^- + CO_3^{2-}]$ was calculated from measured P_{CO_2} values by rearrangement of the Henderson–Hasselbalch equation using pK_1' and αCO_2 values of 6·04 and $0{\cdot}0352\,mmol\,l^{-1}\,Torr^{-1}$, respectively.

Inactive haemolymph acid–base status in crabs carrying shells (Table 1) was typical of other decapods (McMahon & Wilkens, 1983). Interpolating mean Pa_{CO_2} and Pv_{CO_2} (2·5 and 2·7 Torr) onto the oxygenated and deoxygenated CO_2 dissociation curves (Fig. 1A) provided for the theoretical release of $1{\cdot}9\,mmol\,CO_2\,l^{-1}$ *via* the Haldane effect, assuming that postbranchial blood was air-equilibrated and became completely deoxygenated at the tissues. The existing P_{O_2} gradient, however, was only 4 Torr (see Table 3 below), reducing the magnitude of the Haldane effect to $0{\cdot}04\,mmol\,CO_2\,l^{-1}$. Haemolymph from crabs without shells was significantly acidotic (by 0·25 pH units) with half the control levels of circulating bicarbonate (Table 1); this effect may be attributable to trauma (see Wheatly *et al.* 1985).

Sustained exercise caused significant changes in acid–base status (Table 1). pH decreased on average by 0·4 units; P_{CO_2}, $[HCO_3^- + CO_3^{2-}]$ and [lactate] increased by 9-, 3- and 3- to 4-fold, respectively. Post/prebranchial differences in pH, P_{CO_2} and $[HCO_3^- + CO_3^{2-}]$, which were originally $-0{\cdot}05$ pH units, +0·2 Torr and $+0{\cdot}3\,mequiv\,l^{-1}$, increased to $-0{\cdot}07$ pH units, +4·8 Torr and $+1{\cdot}1\,mequiv\,l^{-1}$ after exercise. When Pa_{CO_2} and Pv_{CO_2} values were interpolated onto the *in vitro* CO_2 dissociation curves, the functional Haldane effect increased to $0{\cdot}3\,mmol\,CO_2\,l^{-1}$. Unloaded crabs accumulated less lactate but otherwise experienced a comparable acid–base disturbance.

Acid–base balance was re-established within 1 h after exercise irrespective of access to external sea water at this time (Table 1). Although P_{CO_2} remained significantly elevated it had substantially recovered from exercised levels. P_{CO_2} levels were significantly lower in crabs recovering without water. Changes in postbranchial acid–base status are summarized graphically in Fig. 2. The non-bicarbonate buffering line from Fig. 1C is superimposed. The exercise acidosis is due largely to an increase in respiratory CO_2 which is partially offset by accumulation of metabolic HCO_3^-. Changes in the concentration of metabolic H^+ buffered in the haemolymph (ΔH^+m) were calculated according to McDonald, McMahon & Wood (1979) and correlated with changes in [lactate]. An H^+ deficit of $10\,mequiv\,l^{-1}$ was apparent at the end of exercise (i.e. $\Delta lactate > \Delta H^+m$). During recovery, [lactate] decreased more rapidly, leaving an excess of metabolic H^+.

Table 1. *The major determinants of acid–base status in post- and prebranchial haemolymph in* Coenobita compressus

	Postbranchial			Prebranchial			
Condition	pHa	Pa_{CO_2} (Torr)	Calculated $[HCO_3^- + CO_3^{2-}]_a$ (mequiv l^{-1})	pHv	Pv_{CO_2} (Torr)	Calculated $[HCO_3^- + CO_3^{2-}]_v$ (mequiv l^{-1})	$[lactate]_v$ (mmol l^{-1})
Inactive							
+shells	7·78 ± 0·02 (8)††	2·5 ± 0·6 (8)	5·0 ± 0·7 (8)†	7·73 ± 0·04 (6)††	2·7 ± 0·5 (6)	5·3 ± 0·5 (5)	1·8 ± 0·6 (5)
−shells	7·53 ± 0·03 (3)	2·6 ± 0·4 (3)	2·9 ± 0·6 (3)	7·47 ± 0·05 (3)	2·6 ± 0·7 (3)	3·0 ± 0·9 (3)	2·0 ± 0·3 (3)
Active							
+shells	7·39 ± 0·04 (8)**	22·3 ± 3·0 (8)**	18·0 ± 1·8 (8)**	7·32 ± 0·02 (10)**	27·1 ± 2·9 (10)**	19·1 ± 1·9 (10)**	10·1 ± 1·9 (5)**†
−shells	7·45 ± 0·03 (6)	25·7 ± 6·5 (6)**	24·1 ± 2·2 (6)**	7·36 ± 0·02 (5)	34·3 ± 5·3 (6)**	27·3 ± 4·7 (6)**	4·9 ± 0·7 (3)*
Recovery (+shells)							
+water	7·51 ± 0·13 (4)	5·1 ± 0·8 (4)*†	5·4 ± 0·8 (4)	7·43 ± 0·16 (3)	5·1 ± 0·8 (4)*†	4·6 ± 0·6 (4)†	4·5 ± 1·8 (6)
−water	7·65 ± 0·04 (3)	2·6 ± 0·4 (3)	3·8 ± 0·4 (4)	7·55 ± 0·03 (4)	1·7 ± 0·5 (2)	2·0 ± 0·3 (4)	5·1 ± 2·1 (4)

Measurements were made on inactive crabs and after sustained voluntary activity (150 min at 0·6 cm s^{-1}) in animals running with or without shells. Values are reported after 1 h of recovery either with or without access to 20 ml of 10 % sea water.

Except for inactive (+shells), post- and prebranchial data are paired observations.

*(*) denotes significant differences from control (inactive) value at 5 % (or 1 %) level.

†(†) denotes significant differences between crabs with or without shells and recovery with or without water at 5 % (or 1 %) significance level.

Acid–base exchange with external water

Although inactive hermit crabs exchange their branchial/shell water with an external source, there is no net exchange of acidic equivalents since an ammonia efflux of $440 \pm 100\,\mu equiv\,kg^{-1}\,h^{-1}$ is countered by an approximately equivalent uptake of titratable acidic equivalents (Fig. 3). Following exercise, however, this was a major route for H^+ excretion at rates of $4800 \pm 500\,\mu equiv\,kg^{-1}\,h^{-1}$ in crabs carrying shells ($2800 \pm 350\,\mu equiv\,kg^{-1}\,h^{-1}$ without shells). In both cases, 80% of

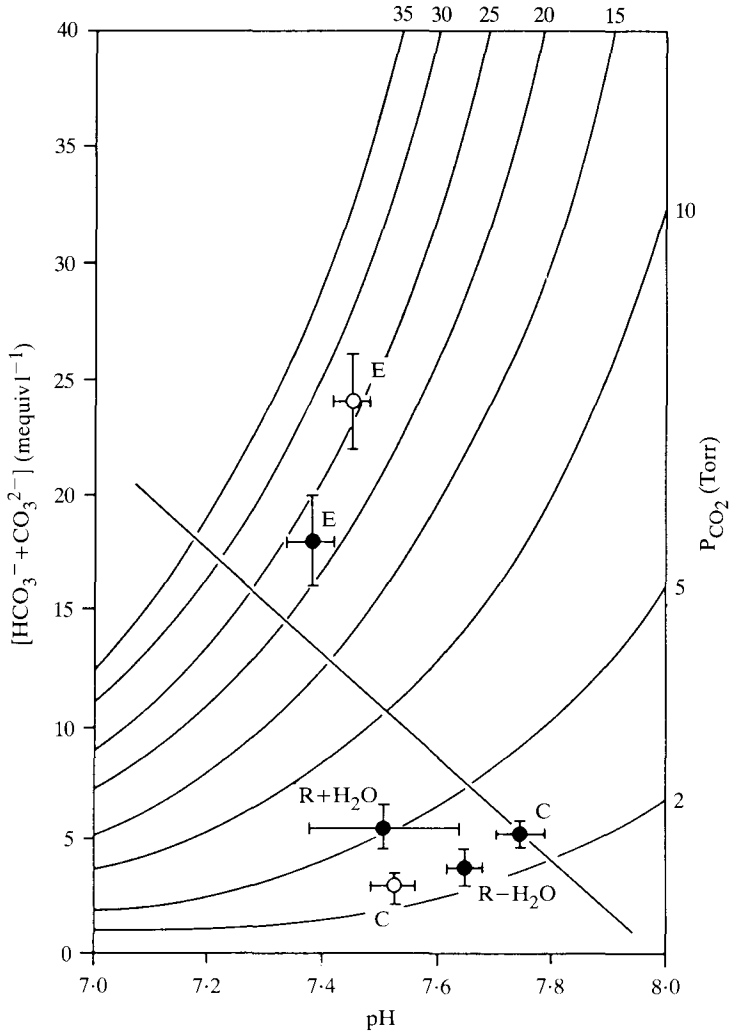

Fig. 2. $[HCO_3^- + CO_3^{2-}]$ *vs* pH diagram for *in vivo* measurements. Non-bicarbonate buffer line taken from Fig. 1C. C, E and R refer to control, exercised and recovered hermit crabs either carrying (●) or not carrying (○) shells and recovering with ($+H_2O$) or without ($-H_2O$) access to water. Data taken from Table 1 for postbranchial haemolymph only. Isopleths for P_{CO_2} were constructed using pK_1' and αCO_2 values of 6·04 and $0{\cdot}0352\,mmol\,l^{-1}\,Torr^{-1}$, respectively.

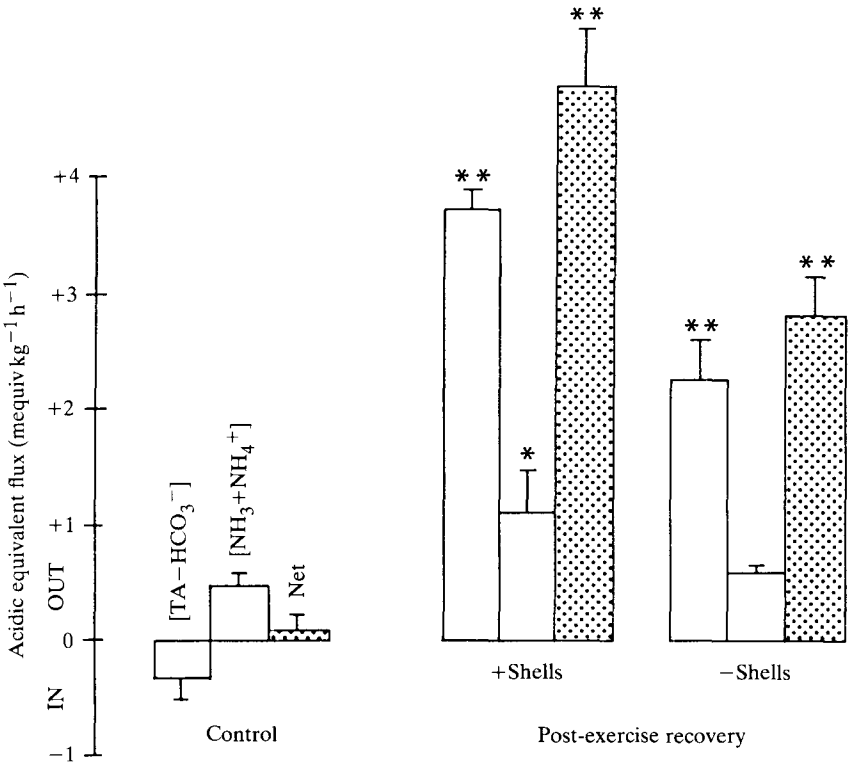

Fig. 3. The titratable [TA−HCO_3^-] and non-titratable [NH_3+NH_4^+] components of the total acidic equivalent flux between hermit crabs and an external source of 10% sea water under control conditions and in the 1-h period immediately following prolonged exercise with and without shells (positive values indicate acidic equivalent output by the animal).

the acidic equivalents were excreted in a titratable form. Ammonia excretion doubled in crabs carrying shells.

Electrolyte status

Electrolyte levels were significantly higher in inactive crabs removed from their shells (Table 2). In animals transporting shells, [Na^+] was just elevated after exercise ($P = 0{\cdot}05$) but all other levels were unchanged. This had the effect of raising the [Na^+]:[Cl^-] ratio from 0·75 to 0·85. Control values were re-established rapidly during recovery if water was available; otherwise levels rose further. Since [Na^+] and [Cl^-] were both now significantly elevated, the ratio between the two was restored.

Oxygen transport

In vitro *O_2-binding characteristics*

The $C_{O_2}^{max}$ of *Coenobita* haemolymph was $1{\cdot}51 \pm 0{\cdot}07$ mmol l^{-1} ($N = 11$); of this, saturated HCy ($C_{HCyO_2}^{max}$) transported $1{\cdot}37 \pm 0{\cdot}07$ mmol l^{-1}. A Bohr shift ($\Delta \log P_{50}/\Delta pH$) of −0·44 was determined (Fig. 4). However, contrary to findings in

other species (e.g. Booth *et al.* 1982; Mangum, 1983*a*), physiological lactate levels (3–13 mmol l^{-1}) had no detectable effect on binding affinity either at low or high P_{CO_2} (Fig. 5). The P_{50} of the combined data was 12 Torr at pH 7·7 and 19 Torr at pH 7·2, corresponding closely with the Bohr plot of Fig. 4.

In vivo *oxygenation*

The *in vitro* equilibrium curves were used to interpolate HCy-bound O_2 concentrations from P_{O_2} and pH measurements made in the field (Table 3; Fig. 6). At mean haemolymph pH (= 7·8), the HCy was saturated at 28 Torr. Post- and prebranchial HCy were 61·8 ± 7·9 % (8) and 37·8 ± 12·3 % (8) saturated, respectively (Fig. 6A). $Ca_{O_2}^{HCy} - Cv_{O_2}^{HCy}$ was 0·329 mmol l^{-1}, accounting for 98 % of the total O_2 delivery. After exercise Pa_{O_2} and Pv_{O_2} both increased, though not significantly. A rightward shift in the O_2 dissociation curve increased $Ca_{O_2} - Cv_{O_2}$ from 0·333 to 0·503 mmol l^{-1} by a depression in prebranchial saturation to 29·3 ± 11·4 % (8).

Acid–base recovery following exercise was the same with or without access to water. Pa_{O_2} was significantly elevated above the exercised level. Pv_{O_2} increased significantly to 26 Torr. Since circulating pH was now restored, these values fell around the shoulder of the O_2 dissociation curve [95·0 ± 7·3 % (4) and 84·0 ± 6·3 % (6) saturated, respectively]. $Ca_{O_2} - Cv_{O_2}$ was half the pre-exercised level (0·124 mmol l^{-1}) and the venous O_2 reserve doubled [1·183 ± 0·087 (4) mmol l^{-1} *versus* 0·530 ± 0·170 (6) mmol l^{-1}].

Crabs removed from their shells (Table 3; Fig. 6B) remained acidotic throughout the experimental procedure. Pre-exercised crabs had a higher mean Pa_{O_2} than animals with shells; correspondingly HCy was saturated [84·0 ± 10·9 % (3)]. Nonetheless, the P_{O_2} gradient across the gill and O_2 content difference were

Table 2. *Electrolyte levels (mean ± S.E.) in prebranchial haemolymph of inactive crabs with and without shells, crabs with shells after activity and recovery with or without access to external water*

	(mequiv l^{-1})				
	Na^+	K^+	Ca^{2+}	Mg^{2+}	Cl^-
Inactive					
+shells	288 ± 18 (6)	11·9 ± 1·0 (6)	21·9 ± 1·0 (6)	55·4 ± 3·2 (6)	404 ± 11 (5)
−shells	501 ± 37 (3)*	21·3 ± 0·7 (3)*	26·6 ± 1·7 (3)*	79·9 ± 6·4 (3)*	671 ± 41 (11)
Active					
+shells	328 ± 10 (10) $P = 0·05$	13·2 ± 1·3 (11)	19·5 ± 1·9 (12)	60·0 ± 3·6 (11)	392 ± 14 (11)
Recovery in animals with shells					
+water	297 ± 23 (4)	11·9 ± 0·5 (4)	19·8 ± 3·1 (4)	51·4 ± 4·8 (4)	399 ± 28 (4)
−water	433 ± 29 (4)*†	13·4 ± 0·4 (4)	21·9 ± 3·4 (4)	70·7 ± 7·7 (4)	549 ± 37 (4)*†

* denotes significant differences (5 % level) between crabs with and without shells or water.
† denotes significant differences (5 % level) from control (inactive) value.

approximately the same. During exercise, tensions and contents both decreased, releasing a similar quantity of O_2 (0·593 mmol l^{-1}) though this time originating from incursion into the venous reserve rather than *via* a Bohr shift in binding affinity.

DISCUSSION

In vitro *CO_2 binding*

The Haldane effect has been described in a number of crustacean and molluscan haemocyanins (Brix, Lykkeboe & Johansen, 1981; Truchot, 1983) and has been quantitatively assessed in relation to the O_2-carrying capacity of the blood. In *Coenobita*, under physiological conditions, 1·22 mmol CO_2 are released for each mmol O_2 bound ($\Delta C_{CO_2}/C^{max}_{HCyO_2}$). This value is double that reported in other decapods (e.g. Truchot, 1976; Randall & Wood, 1981; Booth *et al.* 1982) and is closer to values in cephalopod molluscs (Brix *et al.* 1981), which may relate to the elevated protein concentration. Since the Haldane coefficient represents the amount

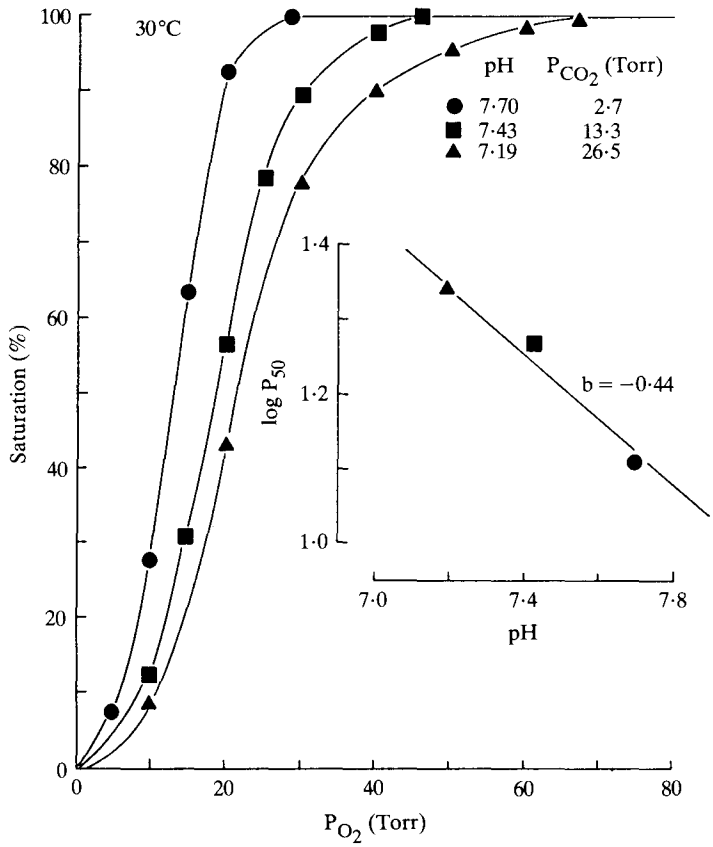

Fig. 4. Oxygen equilibrium curves determined *in vitro* at a range of equilibration pH on pooled *Coenobita compressus* serum. Inset shows the relationship between haemocyanin O_2 affinity (P_{50}:Torr) and pH, where b represents the Bohr value ($\Delta \log P_{50}/\Delta pH$). Regression equation: $\log P_{50} = 4{\cdot}54 - 0{\cdot}44pH$ ($r = 0{\cdot}983$; $N = 3$).

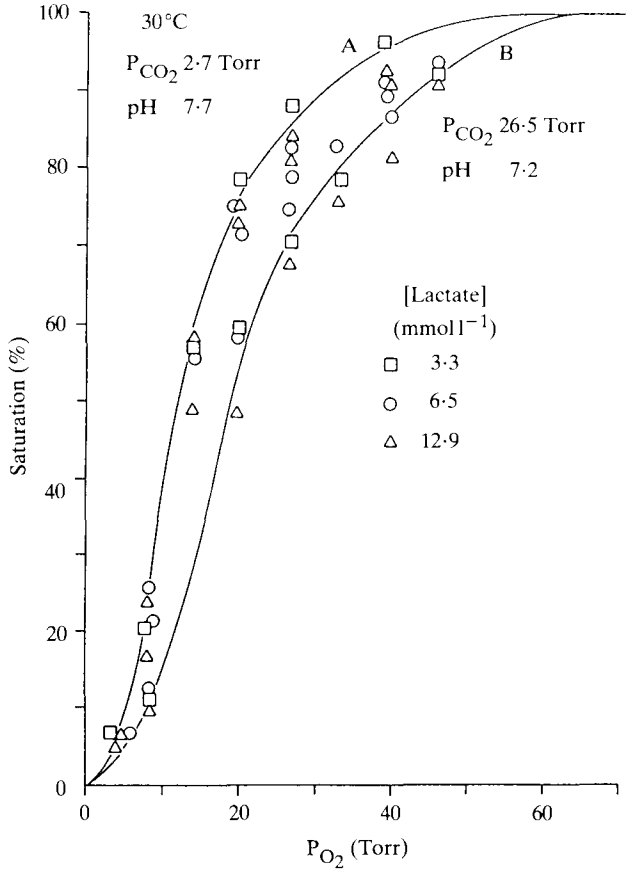

Fig. 5. *In vitro* O_2 equilibrium curves showing the effect of lactate concentration on haemocyanin O_2 affinity at (A) $P_{CO_2} = 2{\cdot}7$ Torr, (B) $P_{CO_2} = 26{\cdot}5$ Torr.

of protons liberated during oxygenation, it should theoretically correspond to the Bohr effect, which reflects the quantity of oxygen liberated by proton binding. The Bohr factor ($\Delta \log P_{50}/\Delta pH$), however, was only $-0{\cdot}44$. When non-correspondence has been previously reported (e.g. Truchot, 1976; Brix *et al.* 1981) the Bohr factor is generally larger, which can be explained if CO_2 is not the only source of protons. The only explanation we can offer in the present case is a non-linear relationship between O_2 saturation and proton release; this effect has been substantiated in human haemoglobin. Values for β and $C^{max}_{HCyO_2}$ are greater than those found in the related species *C. clypeatus* (McMahon & Burggren, 1979), making them the highest so far reported in decapods (McMahon & Wilkens, 1983). The correlation between these two indices agrees well with equations determined by Truchot (1976) and Wood & Randall (1981*b*).

In vivo *acid–base balance*

Inactive P_{CO_2} (Table 1) is atypically low for a terrestrial decapod (see table 1 of Cameron, 1986) but resembles levels recorded in air in the intertidal crab, *Carcinus*

Table 3. *Post- and prebranchial O_2 tensions, total and haemocyanin-bound O_2 contents of pre-exercised and exercised crabs with and without shells and of crabs with shells on recovery with and without access to water*

	Pa_{O_2} (Torr)	Ca_{O_2} (mmol l^{-1})	$Ca_{O_2}^{HCy}$ (mmol l^{-1})	Pv_{O_2} (Torr)	Cv_{O_2} (mmol l^{-1})	$Cv_{O_2}^{HCy}$ (mmol l^{-1})
Pre-exercised						
+shells	13·9 ± 1·1 (8)	0·863 ± 0·109 (8)	0·848 ± 0·108 (8)	10·0 ± 1·9 (6)	0·530 ± 0·170 (6)	0·519 ± 0·168 (6)
−shells	25·3 ± 2·9 (3)**	1·181 ± 0·152 (3)	1·153 ± 0·150 (3)	21·0 ± 1·4 (2)*	0·867 ± 0·108 (2)	0·844 ± 0·106 (2)
Exercised						
+shells	29·4 ± 9·7 (7)	0·901 ± 0·203 (8)	0·869 ± 0·196 (8)	14·9 ± 3·0 (10)	0·419 ± 0·159 (10)	0·402 ± 0·156 (9)
−shells	20·0 ± 2·2 (6)	0·848 ± 0·162 (6)	0·826 ± 0·160 (6)	11·7 ± 1·6 (6)†	0·307 ± 0·078 (6)†	0·278 ± 0·077 (6)†
Recovered						
+shells +water	28·0 ± 3·6 (4)††	1·335 ± 0·238 (4)	1·304 ± 0·090 (4)	26·5 ± 2·1 (2)††	1·183 ± 0·087 (4)†	1·153 ± 0·087 (4)†
+shells −water	31·3 ± 2·7 (3)††	1·399 ± 0·009 (4)††	1·365 ± 0·009 (4)††	26 (1)	1·265 ± 0·017 (4)††	1·236 ± 0·017 (4)††

* denotes significant differences (at 5 % level) between crabs with and without shells or water in the same experimental treatment.
† denotes significant differences (at 5 % level) with control group.
** and †† denote significant differences at 1 % level.

(Taylor & Butler, 1978). Elimination of CO_2 *via* the water reservoir may explain this as well as the low apparent respiratory quotient previously measured in the gas phase (Wheatly *et al.* 1985). For shell water to serve as a CO_2 sink, it requires consistent convection which is achieved in the land crab *Cardisoma* by the action of the flabellae, gill movements and pulsations of the branchial chamber wall (Wood & Randall, 1981*a*).

Hermit crabs experienced similar changes in haemolymph pH and P_{CO_2} after 3 h of voluntary activity to those seen in other land crabs after a 10-min burst of high-speed activity (Smatresk *et al.* 1979; Wood & Randall, 1981*b*). The inability of exercising crabs to excrete CO_2 may be associated with loss of shell water which was noticeably spilled inside the respirometer. The acidosis was primarily attributable to respiratory hypercapnia (Fig. 2). [Lactate] increased during the O_2 deficit early in the exercise period (Table 1) but did not accumulate progressively (M. G. Wheatly, unpublished

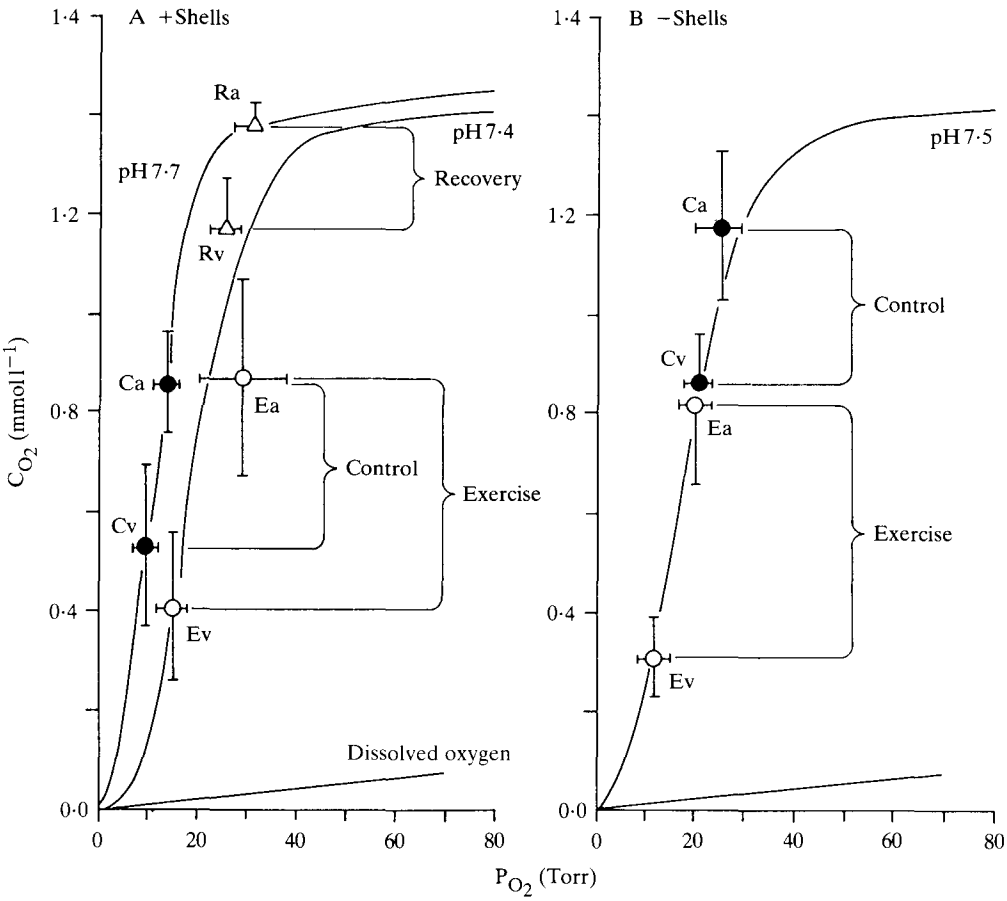

Fig. 6. Mean P_{CO_2} and C_{O_2} of post- (a) and prebranchial (v) haemolymph measured *in vivo* in settled animals (C, ●), after a bout of sustained exercise (E, ○) and 1 h into recovery (R, △; data for recovery with and without access to water were combined). (A) Crabs carrying shells; (B) crabs without shells. *In vitro* dissociation curves taken from Fig. 4 at physiological pH.

observations). This suggests that exercise was fuelled aerobically, which correlates with the short lag time in $\dot{M}_{O_2}$ kinetics, small O_2 deficit and a steady-state $\dot{M}_{O_2}$ capable of fulfilling the total energy requirement (see Wheatly *et al.* 1985), characteristics which are diagnostic of sustained submaximal locomotion in homeothermic vertebrates and insects (e.g. Cerretelli, Pendergast, Paganelli & Rennie, 1979). The extended time course in the present study enabled the initial hypercapnia to be partially compensated by metabolic bicarbonate accumulation (cf. Cameron, 1978).

A similar H^+ deficit was found after sustained swimming in *Callinectes* (Booth *et al.* 1984) and strenuous activity in other crabs (McDonald *et al.* 1979; Wood & Randall, 1981*b*). It has been variously attributed to differential efflux rates of lactate and protons from exercising muscle, buffering of H^+ by $CaCO_3$ stores, and excretion of protons into the external medium. Intracellular sequestration of H^+ was not examined in the present study and circulating Ca^{2+} remained constant (Table 2), which eliminates the first two possibilities. Proton excretion, however (Fig. 3), could account for the H^+ deficit, assuming that it occurs at the same rate during exercise as measured in recovery. Based on a circulating volume of 30% body weight, a net excretion of 33 μequiv during exercise would account for the measured H^+ deficit. This is an efflux rate of 1257 μequiv $kg^{-1}\,h^{-1}$, which is a quarter of the rate measured during recovery. The reduced efflux rate may arise from water spillage.

The increased ammonia efflux during recovery (Fig. 3) could indicate some impairment during exercise or that proton/amino acid reserves were utilized as metabolic substrate. Additionally, it may reflect changes in NH_3 or NH_4^+ gradients, although circulating levels were not measured in this study. However, since ammonia only accounted for 30% of total acidic equivalent efflux, other counterions were predominantly exchanged. Branchial electroneutral ion exchanges (i.e. Na^+/H^+ or NH_4^+; Cl^-/HCO_3^- or OH^-) have now been well documented in ion and acid–base balance in crustaceans (Cameron, 1986).

Considering that most exercise regimes require 6–8 h for recovery of haemolymph acid–base balance, [lactate] and $\dot{M}_{O_2}$ (McMahon, 1981), it was surprising to discover re-establishment of settled levels within 1 h in *Coenobita*. This may relate to the voluntary nature of the activity, which did not drive animals to any physiological limit, as well as the elevated experimental temperature. Since [lactate] is not typically excreted in decapods and we found no evidence for oxidation ($\dot{M}_{O_2}$ not elevated in recovery, Wheatly *et al.* 1985), one must infer that it was removed by gluconeogenesis. This was the conclusion of a recent study on *Uca* by Full & Herreid (1984).

Electrolyte status

Comparison of electrolyte levels in protected and exposed hermit crabs (Table 2) confirmed the role of the adopted molluscan shell in preventing dehydration from the soft, moist pagurid abdomen (Reese, 1969). A thickening of the abdominal cuticle apparently occurs when glaucothoea of the closely related monospecific coenobitid genus *Birgus* dispense with the ancestral behaviour of shell dwelling or when hermit

crabs are reared for extended periods without shells. $[Na^+]:[Cl^-]$ ratios around 0·75 are typical in other decapods acclimated to hyposaline media (Mantel & Farmer, 1983). Although exercise caused crabs to spill shell water, the resulting increase in $[Na^+]$ (Table 2) may not be a simple consequence of haemoconcentration since $[Cl^-]$ remained at pre-exercised levels, indicating an ability for differential ion regulation, perhaps by transcellular Cl^-/lactate exchange (Jackson & Ultsch, 1982). Since $[Ca^{2+}]$ remained constant, there was no evidence for mobilization of exoskeletal $CaCO_3$ to buffer extracellular acidosis, although this has been seen in other terrestrial species (Henry, Kormanik, Smatresk & Cameron, 1981).

O_2 transport

In vitro *O_2-combining characteristics*

Oxygen-binding characteristics (Fig. 4) were similar to those reported for *C. brevimanus* (McMahon & Burggren, 1980). Compared with other terrestrial crabs, however (e.g. table IV, Mangum, 1983*b*), the Bohr factor is relatively small. Although lactate sensitivity has been demonstrated in other members of the Paguroidea (e.g. *Petrolisthes* and *Emerita*), it is by no means universal. In a recent review of the subject, Mangum (1983*a*) concludes that lactate sensitivity is commonly associated with a large Bohr shift and reliance on anaerobic metabolism – neither of which was observed in the present study.

In vivo *O_2-combining characteristics*

Strategies used by *C. compressus* to increase O_2 delivery during sustained voluntary walking (Table 3; Fig. 6) were similar to those identified in swimming blue crabs (Booth *et al.* 1982). Both contrast with the typical response to intense periods of activity (see review by McMahon, 1981), suggesting that marathon and exhausting exercise are functionally dissimilar. The majority of inactive decapods saturate the pigment so that O_2 delivered to respiring tissues originates from physical solution. During exercise, the pigment increases its role in O_2 delivery as circulating P_{O_2} decreases onto the rising phase of the dissociation curve (Mangum, 1983*b*; McMahon & Wilkens, 1983). $C^{max}_{HCyO_2}$ in terrestrial hermit crabs is 2- to 4-fold higher than in other decapods (see tables III, IV of McMahon & Wilkens, 1983). Binding affinity and haemolymph P_{O_2} are comparable, however, which means that Pa_{O_2} and Pv_{O_2} are located around the P_{50} value on the dissociation curve. The pigment therefore accounts for most of the O_2 released under resting conditions.

Both *Coenobita* and *Callinectes* maintain haemolymph P_{O_2} during sustained exercise on account of a rapid 'on-response' in $\dot{M}_{O_2}$ and steady states of $\dot{M}_{O_2}$ that can completely fuel the activity bout (see Booth *et al.* 1982; Wheatly *et al.* 1985). In *Callinectes*, the Bohr shift is offset by a lactate sensitivity which partially restores the overall HCy transport function. Since $Ca_{O_2}-Cv_{O_2}$ is only increased by 10%, the increase in O_2 delivery is attributed primarily to cardiovascular compensations. In *Coenobita*, the Bohr shift was unopposed and $Ca_{O_2}-Cv_{O_2}$ was

increased 50 % by depressing prebranchial saturation (Fig. 6A). Since $\dot{M}_{O_2}$ increased by threefold (Wheatly *et al.* 1985), the cardiovascular system is similarly implicated in enhancing O_2 transport. Crabs removed from their shells exhibited the response typical of bursting activity (Fig. 6B). Several lines of evidence suggest that pre-exercised crabs were severely traumatized by removal from their shells which resulted in higher circulating values of P_{O_2}.

Resting cardiac output ($\dot{V}b$) was calculated, using the Fick principle, as 274 ml $kg^{-1} min^{-1}$ (taking $\dot{M}_{O_2}$ from Wheatly *et al.* 1985). This is almost double that of other species (McMahon & Wilkens, 1983), which is somewhat unexpected since $\dot{V}b$ generally decreases with $C^{max}_{HCyO_2}$. Problems with this method of estimation have recently been discussed by Taylor (1982). $\dot{V}b$ doubled during sustained exercise in *Coenobita* and increased further still during recovery with access to external water. This suggests that gill perfusion is geared towards gill exchange functions occurring at this time (Fig. 3).

Haemolymph convection requirement ($CR = \dot{V}b/\dot{M}_{O_2}$) was low (3·01 $mmol^{-1}$ O_2) in control animals due to the high $C^{max}_{HCyO_2}$. It increased to 8·11 $mmol^{-1}$ O_2 during exercise. Since the design of the respirometer made it impractical simultaneously to monitor respiratory and cardiac frequencies or ventilatory airflow and gas tensions, the following analysis of gas exchange has incorporated data from previous studies (e.g. Wood & Randall, 1981*a*). Since internal P_{O_2} did not change during exercise, one can assume that the mean O_2 diffusion gradient was constant. Therefore increased $\dot{M}_{O_2}$ must be primarily attributed to more favourable conditions for gas transfer across the respiratory surface. Assuming that branchial gas had the same P_{O_2} as *Cardisoma* (Wood & Randall, 1981*a*), the transfer factor ($\dot{T}_{O_2} = \dot{M}_{O_2}/\Delta P_{O_2}$) increased from 0·7 to 2·5 $\mu mol\ O_2\ kg^{-1}\ min^{-1}\ Torr^{-1}$, which is similar to the increase observed in *Callinectes* (Booth *et al.* 1982). Both are higher than reported during sprinting in *Cardisoma* (Wood & Randall, 1981*a*).

For CO_2 excretion to occur in resting animals, the P_{CO_2} of branchial water must be below 2 Torr. A value halfway between circulating and ambient P_{CO_2} would produce a ΔP_{CO_2} of 1·2 Torr, corresponding to a $\dot{T}_{CO_2}$ value of 34·7 $\mu mol\ CO_2\ kg^{-1}\ min^{-1}\ Torr^{-1}$. Based on diffusivities alone, one would expect $\dot{T}_{CO_2}$ to exceed $\dot{T}_{O_2}$ by a factor of 10-fold. The 50-fold discrepancy would therefore confirm our earlier suspicion that conditions across the branchial epithelium favour CO_2 elimination. It is difficult to estimate changes in CO_2 conductance during exercise without more detailed information on the CO_2 diffusion gradient.

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