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ADAPTATIONS TO A TERRESTRIAL EXISTENCE BY THE ROBBER CRAB *BIRGUS LATRO*

I. AN *IN VITRO* INVESTIGATION OF BLOOD GAS TRANSPORT

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Summary

The gas-transporting properties of the haemolymph of *Birgus latro* L. were investigated *in vitro*. This terrestrial anomuran is restricted in distribution to the tropics, and on Christmas Island inhabits a highly stenothermal environment.

The effect of temperature on haemocyanin oxygen-affinity was pronounced ($\Delta H = -39\,kJ\,mol^{-1}$) and was considered to represent the absence of any specific adaptation to environmental temperature. The Bohr effect was large for a terrestrial decapod ($\phi = -0{\cdot}60$), although reduced at low pH. Changes in [Ca] had a significant effect on oxygen affinity of dialysed haemolymph ($\Delta logP_{50}/\Delta log[Ca] = -0{\cdot}39$) whereas [Mg] had no effect. Increasing, [L-lactate] had a small effect on the oxygen affinity of dialysed haemolymph ($\Delta logP_{50}/\Delta log[lactate] = -0{\cdot}013$) but not whole haemolymph. Dialysis increased oxygen affinity, suggesting the presence of a dialysable component that suppresses affinity. The effect of L-lactate was inhibited in whole haemolymph.

The oxygen affinity of *Birgus* haemolymph was largely insensitive to effector substances, with the possible exception of Ca. In the case of lactate, at least, this was not due to a reduced sensitivity of the haemocyanin, a situation different from that in closely related species.

Carbon dioxide transport was also affected by temperature. *Birgus* haemolymph showed a high nonbicarbonate buffer capacity ($\Delta_{CO_2}/\Delta pH = -16\,mmol\,l^{-1}\,pH\,unit^{-1}$) which could be correlated with a high haemocyanin concentration. It is concluded that terrestrial anomuran decapods depend on mechanical adjustments of ventilation and perfusion, rather than employing direct modulation of haemocyanin function, to optimize oxygen delivery.

Introduction

The anomuran *Birgus latro* is arguably one of the most terrestrial decapod species and is an obligate air-breather. The species has a wide, although

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contracting, distribution throughout the tropical Indo-Pacific region (Harms, 1932). The lungs of *Birgus* are large and have developed by extensive vascularization and evagination of the lining of the branchial chambers (Harms, 1932). Gills are present in *B. latro*, but are of relatively small surface area and seem unlikely to be important in gas exchange (Semper, 1878; Harms, 1932; Cameron, 1981*a*).

The roles of the gills and lungs in gas exchange and acid–base balance in landcrabs are poorly understood and there is little direct evidence concerning their relative contributions. In *Birgus*, the removal of the gills resulted in a small increase in ventilation rate and a slight respiratory acidosis (Smatresk & Cameron, 1981). This would suggest that the gills are not crucial to maintained gas exchange. In view of this, it is probable that many of the functions carried out by gills in aquatic crustaceans may be partitioned between the two exchange organs in *Birgus*. Facultative partitioning of gas exchange has been demonstrated in the amphibious *Holthuisana transversa* in which the gills or the lungs assume ascendency depending on the respiratory medium (Greenaway *et al.* 1983*a*,*b*; Greenaway, 1984; Taylor & Greenaway, 1984).

Despite a number of papers resulting from the *Alpha Helix* expedition to the Palau Islands (e.g. Burggren & McMahon, 1981; Cameron, 1981*b*; Henry & Cameron, 1981; McMahon & Burggren, 1981; Smatresk & Cameron, 1981) our understanding of the respiratory physiology of *Birgus* is far from complete (see also Cameron & Mecklenburg, 1973).

The role of the haemolymph in the respiration of this species is an important area requiring further study. Our knowledge of the role of modulator systems in regulating haemocyanin function in anomurans is limited to a handful of papers. These recent studies have shown that the haemolymph of terrestrial decapods is insensitive to the identified modulator/effector systems and, moreover, this insensitivity probably evolved more than once (Morris & Bridges, 1986*a*; Wheatly *et al.* 1986; Morris *et al.* 1987). With so few data it is impossible to conclude whether this is a general phenomenon. An important part of this study was to clarify this point, as it has fundamental implications in terms of the stress response of the oxygen uptake and delivery system. The more potent haemocyanin modulators include H^+ (Bohr effect) and Mg, Ca and lactate (Mangum, 1983; Bridges & Morris, 1986; McMahon, 1986).

In addition to providing basic data on the function and evolution of haemocyanin, the study was also designed to characterize haemolymph properties essential to a comprehensive study of the respiratory physiology of *Birgus*. The data are discussed in relation to the ecology and behaviour of the animal with reference to the role of the haemolymph in blood gas transport *in vivo* (Greenaway *et al.* 1988).

Materials and methods

Animal collection and sampling

Specimens of *Birgus latro* (250–500 g) were collected under permit from the Australian Territory of Christmas Island (location 10°28′ S, 105°38′ E). The crabs were individually packed and then air-freighted within 3 days of collection to the University of New South Wales, Kensington, NSW, where the investigation was carried out. Mortality during shipment was less than 1 % and no animals died subsequently. The *Birgus* were maintained in a humidified constant-temperature room at 25 °C either in terraria or, prior to an experiment, individually in plastic fish boxes. Fresh fruit and vegetables and dry, fish-based, cat food pellets were provided on a regular basis, together with ample fresh water for drinking. Both food and water were withdrawn 24 h prior to sampling.

Haemolymph samples (1 ml) were taken from the venous sinus at the base of the second walking leg. Blood was taken from 12 animals and sampling required less than 10 s. The samples were then pooled to provide a homogeneous solution allowing comparison of all data. Any clots were disrupted by forcing the blood through a 25 gauge hypodermic needle after which the blood was centrifuged at 10 000 *g* and the supernatant haemolymph stored for up to 4 weeks in 1 ml samples at 4 °C until required. Control determinations showed no discernible change in the functional properties of the haemolymph over this period.

Initial haemolymph determinations

The concentrations of Na, K, Mg, Ca and Cu in the pooled haemolymph were determined using an atomic absorption spectrophotometer (Varian AA5). For the measurement of [Ca] all samples and standards contained $LaCl_3$ (Sparkes & Greenaway, 1984). The concentration of chloride was measured using a chloride titrator (CMT 10, Radiometer, Copenhagen) and the osmotic pressure with a vapour pressure osmometer (Wescor 5100C, Logan, USA). The concentration of L-lactate in the blood and in subsequent haemolymph preparations was determined using the Boehringer test kit (catalogue no. 139084, Boehringer Mannheim GmbH, Mannheim, FRG). This method depends on the conversion of L-lactate to pyruvate by lactate dehydrogenase and the measurement at 339 nm of the concomitant conversion of NAD^+ to NADH. Pyruvate is removed from the reaction mixture by glutamate–pyruvate transaminase.

Construction of oxygen equilibrium curves

Oxygen equilibrium curves were constructed using a spectrophotometric system (Morris *et al.* 1987). All gas mixtures used during the investigation were supplied by Wösthoff mixing pumps (types M 301a/f and SA 18, Wösthoff, Bochum, FRG). The pH of all samples of haemolymph was measured near to the P_{50} oxygen tension using the G299a capillary electrode in a BMS 2 blood microsystem (Radiometer). The pH of the blood was normally varied by changing the CO_2 content of the gas mixture. This method allowed the calculation of the Bohr coefficient ($\Delta \log P_{50}/\Delta pH$) under the various experimental conditions employed. The Bohr coefficient and plots were in all cases calculated by regression analysis of data sets where $r > 0{\cdot}90$. The cooperativity (n_{50}) of haemocyanin–oxygen binding was determined by regression analysis of saturation values between 25 and 75 % in accordance with the Hill equation.

The effect of temperature on haemolymph oxygen-affinity was investigated by constructing equilibrium curves for whole haemolymph at 15, 20, 25, 30 and 35°C. On Christmas Island the mean temperature in the rain forest is approximately 26°C. The change in the heat of oxygenation of the haemolymph (ΔH) accompanying an increase in temperature was calculated according to the equation:

$$\Delta H = 2{\cdot}303R\frac{\Delta \log P_{50}}{\Delta(T^{-1})} \quad (\mathrm{kJ\,mol^{-1}}),$$

where R is the gas constant and T the absolute temperature.

The dependence of oxygen affinity on [Ca] and [Mg] was determined by constructing equilibrium curves for dialysed blood in which the concentration of Ca or Mg had been adjusted. Dialysis of *Birgus* haemolymph was carried out in a Ringer's solution, based on the measured inorganic salt content of the haemolymph, with the following composition (in $\mathrm{mmol\,l^{-1}}$): NaCl, 316; KCl, 9·4; $MgSO_4$, 1; $MgCl_2$, 21; $CaCl_2$, 16·0; $NaHCO_3$, 1. In those cases where the concentrations of Mg or Ca were altered (12 and 46 $\mathrm{mmol\,l^{-1}}$, 8·4 and 24·7 $\mathrm{mmol\,l^{-1}}$, respectively) the concentration of NaCl in the Ringer's solution was adjusted so that [Cl] remained constant.

The specific sensitivity of the haemocyanin oxygen-affinity to CO_2 was investigated using techniques described by Morris *et al.* (1985) in which the CO_2 Bohr shift (pH altered by CO_2) and the fixed-acid Bohr shifts (0·2 and 3 % CO_2, pH controlled with HCl and NaOH) were compared. Bohr coefficients ($\phi = \Delta\log P_{50}/\Delta\log pH$) were calculated by regression analysis and compared using analysis of covariance.

The effect of lactate was investigated in both dialysed and nondialysed haemolymph. Samples of haemolymph containing L-lactate at different concentrations were prepared for the construction of oxygen equilibrium curves using previously described methods (Bridges *et al.* 1984; Morris *et al.* 1985). The concentration of L-lactate was subsequently determined for all preparations and these values are provided in the appropriate figures.

Determination of CO_2 equilibria

The dependence of C_{CO_2} on P_{CO_2} in the haemolymph of *Birgus* was determined at 15, 25 and 35°C using published methods (Morris *et al.* 1985). Briefly, 100 μl samples were equilibrated in a BMS2 blood microsystem (Radiometer) with gas mixtures of varying P_{CO_2} controlled by gas-mixing pumps. The C_{CO_2} was determined according to the method of Cameron (1971). The $[HCO_3^-]$ was calculated using CO_2 solubility coefficients extrapolated for 80 % sea water from the tables of Dejours (1981). Determinations were carried out for both oxygenated ($P_{O_2} > 150$ mmHg; 1 mmHg = 133·3 Pa) and deoxygenated ($P_{O_2} < 1$ mmHg) blood. The dependence of C_{CO_2} on P_{CO_2} was determined in triplicate and pH measured in duplicate. Unless otherwise stated all values are given as means ± 1 S.D.

Results

The concentrations of inorganic elements determined in the pooled sample were in $mmol\,l^{-1}$: Na, 357 ± 15; K, $9{\cdot}4 \pm 1{\cdot}2$; Cl, 348 ± 6; Mg, $18{\cdot}6 \pm 2{\cdot}6$; Ca, $17{\cdot}0 \pm 1{\cdot}4$; and Cu, $3{\cdot}7 \pm 0{\cdot}3$. The osmotic pressure was $752 \pm 48\,mosmol\,kg^{-1}$ and the concentration of L-lactate was $0{\cdot}75\,mmol\,l^{-1}$. From the measured [Cu] a theoretical maximal oxygen-carrying capacity ($Hc{-}O_2^{max}$) of $1{\cdot}85\,mmol\,l^{-1}$ was calculated.

The effect of temperature on O_2 affinity

Increasing temperature markedly reduced the oxygen affinity of *Birgus* haemolymph but did not affect the magnitude of the Bohr coefficient (analysis of covariance, $P < 0{\cdot}01$) which had a value of $-0{\cdot}60 \pm 0{\cdot}04$. The Bohr coefficients calculated for each temperature are shown in Fig. 1. The effect of temperature was not discernibly different for 5 °C intervals throughout the temperature range used and the calculated values of ΔH yielded a mean value of $-38{\cdot}5 \pm 10{\cdot}9\,kJ\,mol^{-1}$. Changes in temperature had no measurable effect on cooperativity (Fig. 1), and the mean n_{50} was $2{\cdot}84 \pm 0{\cdot}28$.

The effect of [Ca] and [Mg]

Neither the Bohr coefficient nor the P_{50} of dialysed *Birgus* haemocyanin was

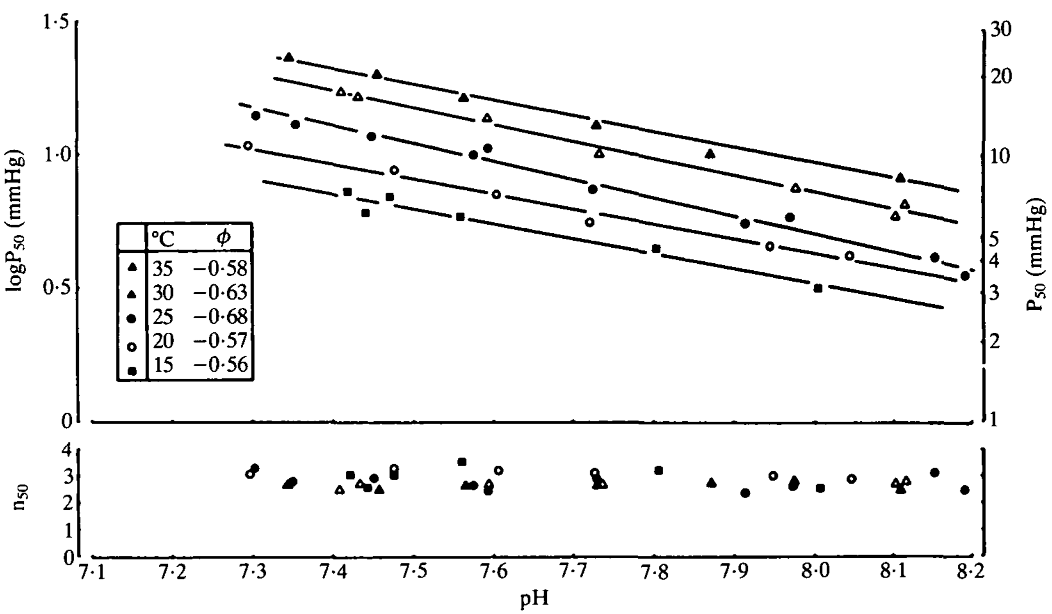

Fig. 1. The effect of temperature on the oxygen affinity of *Birgus latro* whole haemolymph shown as the dependence of $\log P_{50}$ on pH. The slope of this relationship represents the Bohr shift and the Bohr coefficient (ϕ) is shown in the inset. The effect of temperature on the cooperativity of O_2 binding is shown in the lower panel. *N* is as shown and all plots were determined by regression analysis ($r > 0{\cdot}90$).

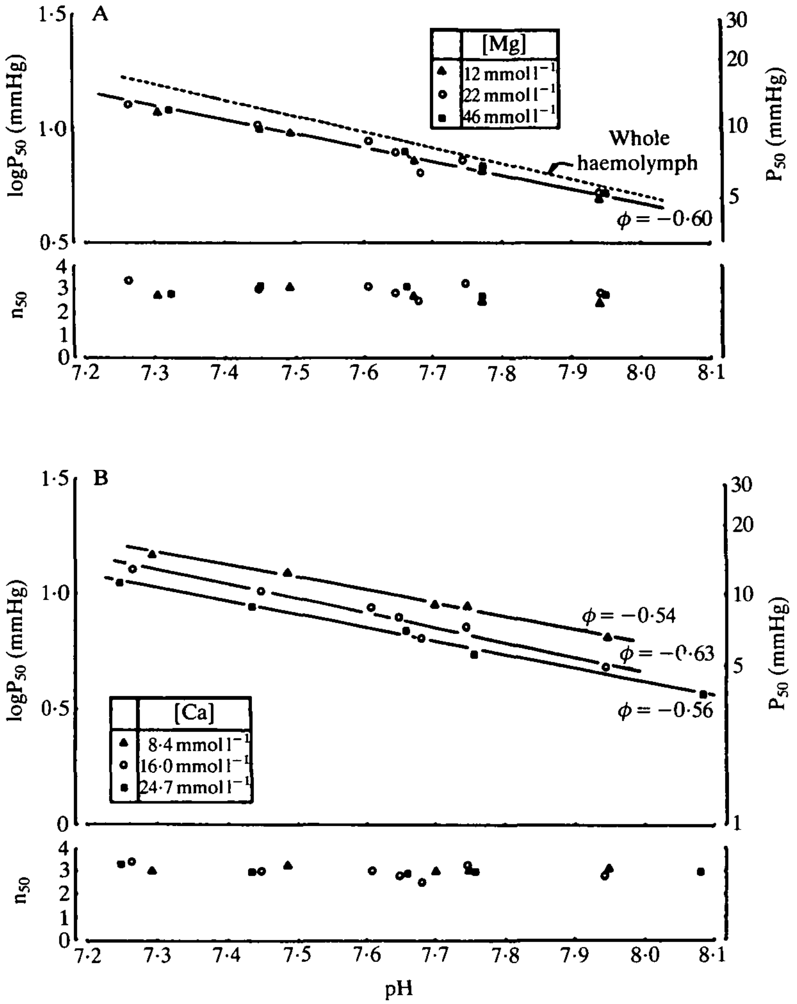

Fig. 2. (A) The oxygen affinity ($logP_{50}$) of dialysed haemolymph from *Birgus latro* at 25 °C containing three different concentrations of Mg over the physiological pH range. The Bohr coefficient (ϕ) is also shown. The broken line gives the Bohr plot calculated for nondialysed haemolymph. The lower panel shows the corresponding cooperativity values. (B) The potentiating effect of Ca on the oxygen affinity ($logP_{50}$) of dialysed *Birgus latro* haemolymph throughout the physiological pH range. The calculated Bohr factors (ϕ) are given for each of the three Ca concentrations. The cooperativity of O_2 binding at different values of [Ca] and pH is shown in the lower panel.

altered by changes in [Mg] within the range 12–46 mmol l^{-1} (Fig. 2A). The combined data could be described by a single equation with a slope of −0·60. Dialysis of the haemolymph induced a small, but significant, increase in oxygen affinity (analysis of covariance, $P<0·05$) but no change in the Bohr factor.

Increases in [Ca] from 8·4 to 16 and from 16 to 24·7 mmol l^{-1} resulted in

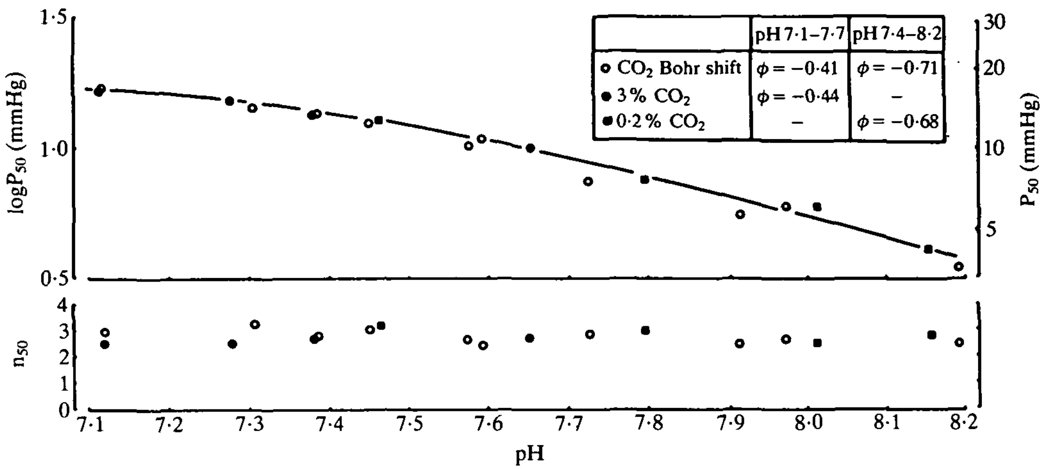

Fig. 3. Comparison of the CO_2 Bohr shift with fixed-acid Bohr shifts in whole haemolymph of *Birgus latro* at 0·2 and 3% CO_2 and 25°C. Owing to the curvilinear nature of $\Delta logP_{50}/\Delta pH$ over the extended pH range used, the Bohr factors were calculated from regression equations over the appropriate reduced ranges shown in the inset. The cooperativity of the various preparations over the extended pH range are shown in the lower panel.

significant increases in oxygen affinity (analysis of covariance, $P < 0.01$) in the absence of a change in the Bohr factor (Fig. 2B). The effect of [Ca] on haemocyanin oxygen-affinity within the pH range used could thus be described by the equation: $logP_{50} = 1.21 - 0.39log[Ca]$ (intercept $\pm$ 0·04, slope $\pm$ 0·12).

The variability of the estimate of cooperativity was greater in the dialysed blood, but the mean value of n_{50}, 3·09 ± 0·85, was not significantly altered compared with that of nondialysed haemolymph or in response to changes in [Mg] and [Ca].

The specific effect of CO_2

A comparison of the CO_2 and fixed-acid Bohr coefficients revealed no specific effect of CO_2 when its concentration in the haemolymph was increased from 0·2 to 3·0% ($\Delta P_{CO_2} = 20.7$ mmHg) (Fig. 3). Over the extended pH range used the slightly curvilinear nature of $\Delta logP_{50}/\Delta pH$ became apparent and therefore comparisons of the Bohr coefficient (ϕ) were made over the appropriate pH ranges (Fig. 3). No dependence of n_{50} on CO_2 concentration could be measured.

The effect of L-lactate

There was no effect of L-lactate on the oxygen affinity of whole haemolymph, but a small effect was measured for dialysed haemolymph (analysis of covariance, $0.10 > P > 0.05$) as indicated in Fig. 4. The increase in haemocyanin oxygen-affinity after dialysis was again evident ($P < 0.05$). The small effect of L-lactate was

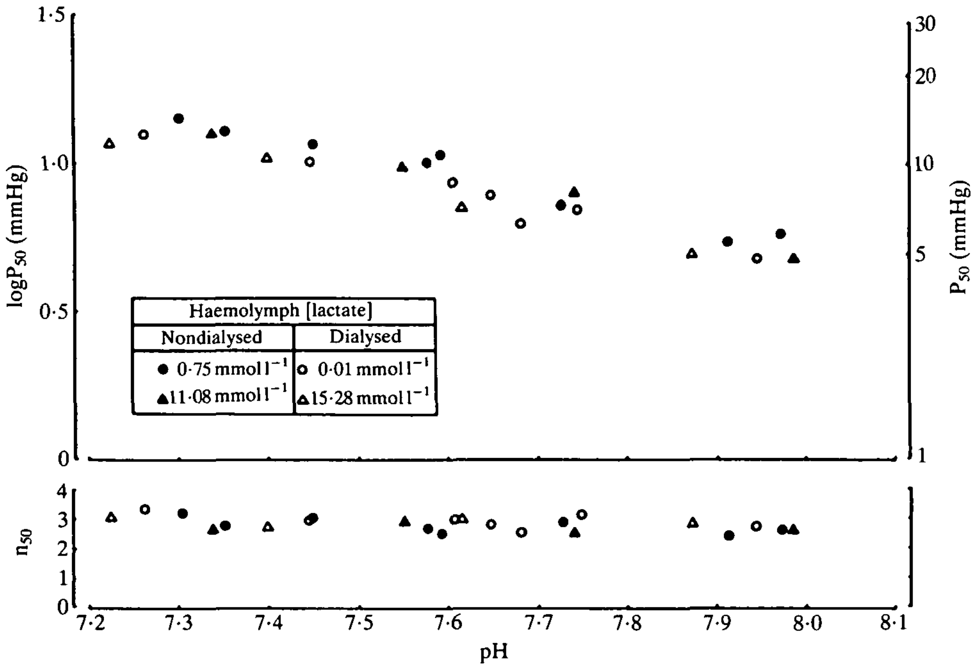

Fig. 4. The dependence of oxygen affinity ($logP_{50}$) of dialysed and nondialysed haemolymph of *Birgus* on the concentration of L-lactate over the physiological pH range. Cooperativity (n_{50}) values are shown in the lower panel.

determined at pH 7·8 as $\Delta logP_{50}/\Delta log[lactate] = -0{\cdot}013$. Again there was no discernible effect on the cooperativity of oxygen binding, $n_{50} = 2{\cdot}80 \pm 0{\cdot}19$.

The effect of temperature on haemolymph CO_2 equilibrium

The relationships between C_{CO_2} and P_{CO_2} in the haemolymph of *Birgus* at 15, 25 and 35 °C are shown in Fig. 5. There was a tendency for CO_2 capacity to increase at lower temperatures above that dictated by the increased solubility of CO_2, indicating increased Hc–CO_2 association. The CO_2 equilibria presented no evidence in support of a Haldane effect. Combining data from oxygenated and deoxygenated blood, the dependence of pH on temperature ($\Delta pH/\Delta T$) was calculated as $-0{\cdot}006\,mmol\,l^{-1}\,pH\,unit^{-1}$ (P_{CO_2} varied by 0·11 mmHg).

The $[HCO_3^-]$ calculated from these data are shown in Fig. 6 which shows the effects of temperature more clearly. A temperature increase from 15 to 25 °C elicited a decrease in HCO_3^- capacity of $>3\,mmol\,l^{-1}$ at constant pH, without altering the nonbicarbonate buffer power of the haemolymph which averaged $-15{\cdot}3\,mmol\,l^{-1}\,pH\,unit^{-1}$. An increase by a further 10 °C to 35 °C resulted in a significant increase in the buffer power to $-20{\cdot}3\,mmol\,l^{-1}\,pH\,unit^{-1}$. It was unlikely that this result represented haemoconcentration due to evaporation of the haemolymph, as a fresh 100 μl sample was equilibrated for each determination and the gas mixtures used were humidified. Comparing deoxygenated and oxygenated blood at constant pH confirmed the absence of any significant Haldane effect.

Discussion

There are few reports in the literature on the physiology of anomuran haemocyanins (e.g. Morris & Bridges, 1986*a*; Wheatly *et al.* 1986). That of *Birgus* was investigated, however, by Burggren & McMahon (1981) who reported P_{50} values (see also McMahon, 1986). Their reported value of a P_{50} of 21 mmHg at 30°C (pH 7·6) is lower affinity than that reported for the same temperature in the present study (13·6 mmHg, pH 7·6). The difference could be due to variations between the Indian and Pacific Ocean populations. However, a single value of 14·5 mmHg (28°C, pH 7·5) reported by Cameron & Mecklenburg (1973) for *Birgus* from the Marshall Islands agrees with the data presented here. These values represent, however, a moderately low oxygen affinity fairly typical of

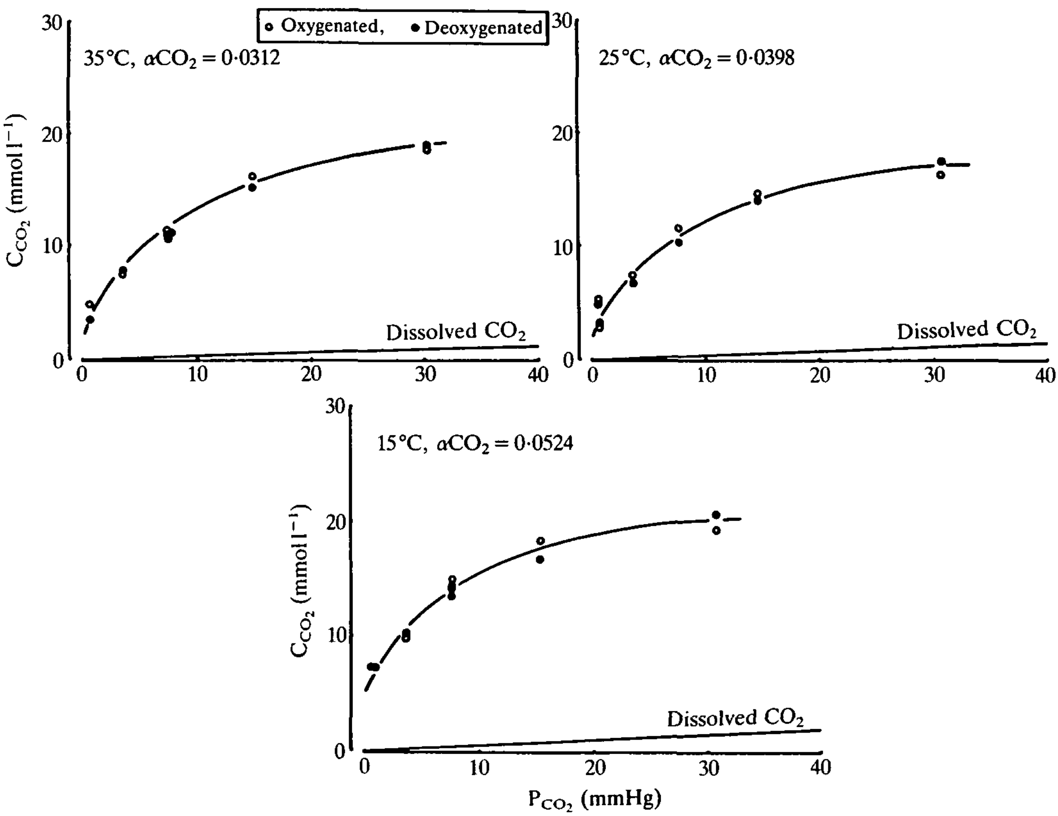

Fig. 5. Carbon dioxide equilibria for oxygenated and deoxygenated *Birgus latro* haemolymph at three different temperatures. The CO_2 solubility coefficients are for 80 % sea water. Each equilibrium curve was constructed in triplicate. The values shown are means, in all cases the error was smaller than the symbol used. Note that where values were coincident each has been slightly displaced for clarity.

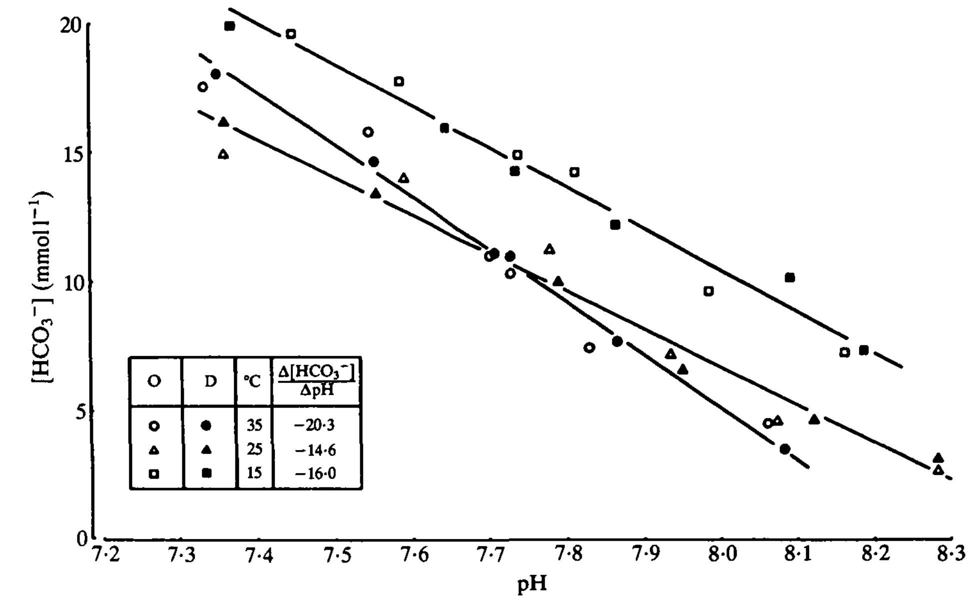

Fig. 6. The $[HCO_3^-]$/pH diagram for oxygenated (open symbols) and deoxygenated (closed symbols) whole haemolymph of *Birgus latro* at three different temperatures calculated using data in Fig. 5. The P_{CO_2} isopleths are omitted owing to the temperature differences. The buffer power ($\Delta[HCO_3^-]/\Delta pH$) is given for each case in the inset. The pH values are the means of duplicates.

terrestrial decapods, anomurans particularly, and suggest that the pigment functions as more than a simple store for O_2 (for reviews see Mangum, 1983; McMahon & Burggren, 1988; see also Greenaway *et al.* 1988). The calculated oxygen-carrying capacity of 1·85 mmol l^{-1} is close to the maximum recorded value for a decapod (Mangum, 1983). It is likely that using [Cu] to estimate oxygen-carrying capacity leads, however, to an overestimate of haemocyanin concentration and O_2-carrying capacity, as measured arterial C_{O_2} was lower than that derived from [Cu] (1·32 mmol l^{-1}) (Greenaway *et al.* 1988). According to the analysis of McMahon & Wilkens (1983), this high oxygen content may be correlated with a low, mass-specific, cardiac output and therefore represents an energetic saving. The calculated Bohr effect in *Birgus*, although relatively modest in comparison to some aquatic species, is large for a terrestrial species (for reviews see Mangum, 1983; McMahon & Burggren, 1988). Another estimate of the Bohr coefficient of −0·90 for Pacific *Birgus*, discussed by McMahon (1986) and McMahon & Burggren (1988), supports this conclusion.

The effect of temperature

Increased temperature significantly reduced the oxygen affinity of *Birgus* haemocyanin. Few higher ΔH values ($-39\,kJ\,mol^{-1}$) have been recorded for crustacean haemocyanin (Bridges, 1986; Burnett *et al.* 1988). In addition, the sensitivity was uniform over the temperature range used, a situation which differs from that reported for both the land hermit crab *Coenobita clypeatus* (Morris & Bridges, 1986*a*) and the terrestrial brachyuran *Ocypode saratan* (Morris & Bridges, 1986*b*). In contrast, the terrestrial/freshwater crab *Holthuisana transversa* exhibits a response similar in many ways to that of *Birgus* (Morris *et al.* 1987). In the rain forests of Christmas Island diurnal and seasonal temperature variations are only 2–3°C. Under these conditions it is difficult to imagine that there is any selective pressure for these crabs to adapt to extreme temperatures. The accumulating published data on the O_2–haemocyanin temperature response now suggest that those species inhabiting stenothermal environments, or having behavioural mechanisms to avoid temperature extremes, exhibit no reduced temperature-sensitivity at temperatures near the environmental mean.

The effects of [Mg] and [Ca]

Both Mg and Ca are known to affect the oxygen affinity of most crustacean haemocyanins (see Introduction) and Ca has previously been suggested to be an effector of *Birgus* haemocyanin (McMahon, 1986), but until now this has been unsubstantiated. Recent investigations suggest that the haemocyanins of terrestrial crabs are insensitive to effector substances (Morris & Bridges, 1986*a*,*b*; Wheatly *et al.* 1986; Morris *et al.* 1987). Although the insensitivity to [Mg] supports this conclusion, oxygen binding by the haemocyanin of *Birgus* exhibits a marked Ca-sensitivity. Truchot (1975) showed that the haemocyanin of *Carcinus maenas* was sensitive to [Ca] ($\Delta \log P_{50}/\Delta \log[Ca] = -0{\cdot}28$) and Mason *et al.* (1983) demonstrated a high sensitivity for *Callinectes sapidus* (−0·82). The measured value for *Birgus* (−0·39) might therefore be important. Burggren & McMahon (1981) demonstrated that 3–4 days of dehydration could result in 14 % loss in body mass and as much as 50 % increase in the [Ca] in the haemolymph of *Birgus*. Observations on Christmas Island indicated that the crabs drank frequently from hollows in tree roots (rain water) and from ground water ($3\,mmol\,l^{-1}$ Ca) when available. Dehydration and the fluctuating [Ca] of drinking water may result in varying haemolymph [Ca] and thereby alter oxygen affinity. Whether this is the case, or indeed is of any adaptive significance, is unclear.

The effect of L*-lactate*

As with [Ca], physiological variations in [L-lactate] are probably not important in affecting the oxygen affinity of haemocyanin in anomuran crabs (Morris & Bridges, 1986*a*; Wheatly *et al.* 1986). A small lactate effect was measured in the dialysed haemolymph of *Birgus* but, for several reasons, must be considered to be of dubious physiological significance. First, a coefficient of −0·013 is relatively small (cf. Bridges & Morris, 1986) and in *Birgus* haemolymph at pH 7·8 an increase in [lactate] of $10{\cdot}3\,mmol\,l^{-1}$ increased O_2 affinity by only 0·6 mmHg. However, *Birgus* produces exceptionally large amounts of L-lactate (Greenaway *et al.* 1988). Second, this effect of L-lactate was not apparent in whole haemolymph. Dialysis of

Birgus haemolymph increased affinity and may also have removed an inhibitor of the potentiating effect of L-lactate. Recently identified modulators such as urate, which are known to accumulate in terrestrial decapods, including *Birgus* (Gifford, 1968; Henry & Cameron, 1981), have a secondary effect of diminishing the response to lactate (Morris *et al.* 1986; Morris & Bridges, 1986*c*). It would seem possible that a similar effect is responsible for the inhibition of the lactate effect in the haemolymph of *Birgus*.

The specific effect of CO_2

The oxygen affinity of *Birgus* haemocyanin is insensitive to P_{CO_2} changes within the physiological range. Severe exercise in *Birgus* results in depression of haemolymph C_{CO_2} by up to 50 %, usually accompanied by some increase in P_{CO_2} (Smatresk & Cameron, 1981; Greenaway *et al.* 1988). A potentiation of oxygen affinity by P_{CO_2} is unlikely to be beneficial under these conditions. Therefore, there would seem to be little selective pressure to promote such an effect in an air-breather, and the *in vivo* data indicate that it is not required (Greenaway *et al.* 1988).

The effect of temperature on haemolymph CO_2 equilibria

Temperature increases have the predictable effect of decreasing haemolymph CO_2 capacity but often to an extent beyond that due to the decreased solubility of the gas (Morris *et al.* 1985; Taylor *et al.* 1985; Morris *et al.* 1987). The effect of temperature change on CO_2 equilibria in *Birgus* haemolymph between 15 and 25 °C conforms to the normal pattern. Between 25 and 35 °C this is true only at high pH owing to an apparent increase in the nonbicarbonate buffer capacity of the haemolymph. The physiological importance of these latter data should be treated with some reservation until a corroborative study can be carried out.

The buffer power of the haemolymph was high at all temperatures, corresponding to a high haemocyanin concentration (140 mg ml^{-1} calculated from [Cu]; approx. 110 mg ml^{-1} calculated from Hc–O_2^{max} assuming haemocyanin $M_r = 75\,000$). The importance of the small amount of nonrespiratory protein normally present in crustacean haemolymph will be minimal in the presence of large amounts of haemocyanin. The measured $\Delta C_{CO_2}/\Delta pH$ of $-15{\cdot}8$ mmol l^{-1} pH unit^{-1} was smaller than the value reported by Smatresk & Cameron (1981) ($-22{\cdot}7$ mmol l^{-1} pH unit^{-1}), although these workers report resting *in vivo* pH values similar to those of *Birgus* from Christmas Island (Greenaway *et al.* 1988). Considering the large amount of metabolic acid released into the extracellular space, it is likely that the large amount of haemocyanin has a second, important role as a buffer substance. The value reported by Cameron (1981*b*), $-16{\cdot}0$ mmol l^{-1} pH unit^{-1}, was essentially the same as that measured in the present study.

There was no detectable Haldane effect. Although the haemolymph was almost completely deoxygenated and resaturated during a single circulation in exercising *Birgus*, this apparently played no role in the excretion of CO_2. Linkage between the Bohr and Haldane effects implies that it would be impossible to evolve a significant oxygenation-dependent CO_2 capacity without increasing the pH sensitivity of oxygen binding above that found here for *Birgus* haemolymph. Such enhanced pH sensitivity of oxygen binding may compromise oxygen loading in a terrestrial species experiencing severe haemolymph acidosis.

In conclusion, the haemocyanin of *Birgus* is present at high concentration in the haemolymph leading to high O_2 and CO_2 capacities as well as relatively elevated buffer capacity. Modulator-insensitive haemocyanins in terrestrial decapods probably evolved independently several times, leading to an increased dependence of maintained oxygen delivery on mechanical/morphological adaptations. The absence of lactate-sensitivity in *Birgus* haemolymph was not due only to the sensitivity of the haemocyanin but also to the inhibition of the lactate effect. Whether the Ca effect is reduced or inhibited in whole compared with dialysed haemolymph is as yet undetermined. Techniques different from those used in the present study would be required to clarify this. Temperature is the major modifying influence on haemocyanin oxygen-affinity, even though these animals occupy stenothermal habitats. A marked Bohr shift at resting pH becomes much less significant at the low pH levels seen in exercised *Birgus*, reducing the importance of this phenomenon. *Birgus* haemocyanin functionally resembles that of other terrestrial decapods but somewhat different mechanisms may be involved. Modulation of *Birgus* haemolymph, as with other investigated terrestrial species, appears to be unimportant, suggesting that air-breathing crabs rely primarily on ventilatory and perfusion adjustments to maintain oxygen supply to the tissues.

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