J. exp. Biol. 140, 493–509 (1988)
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ADAPTATIONS TO A TERRESTRIAL EXISTENCE BY THE ROBBER CRAB *BIRGUS LATRO*

II. *IN VIVO* RESPIRATORY GAS EXCHANGE AND TRANSPORT

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Accepted 9 June 1988

Summary

The lungs are responsible for essentially all uptake of oxygen in *Birgus latro*. Elimination of CO_2 in resting crabs appears to occur largely across the gills but during exercise approximately half the output of CO_2 is pulmonary. Pa_{O_2} was high in resting crabs (43·8 mmHg; 1 mmHg = 133·3 Pa) but fell during exercise (to 27 mmHg). Pv_{O_2} remained constant at 10–12 mmHg. Pa_{CO_2} rose substantially during exercise (from 7·1 to 14·6 mmHg).

Haemocyanin delivered 90 % of oxygen in resting crabs rising to 97 % following exercise. Oxygen delivery at rest was 0·46 mmol l^{-1} haemolymph rising to 0·72 mmol l^{-1} following exercise. Pigment-bound oxygen capacity was 1·1 mmol l^{-1}. Oxygen delivery to the tissues was diffusion-limited during exercise.

Anaerobic metabolism during exercise raised the concentration of L-lactate in the haemolymph 100-fold (from 0·25 to 25 mmol l^{-1}) and concomitantly caused a fall in pH of 0·7 units. This acidosis was partially compensated by the end of the 30-min exercise period.

Introduction

Birgus latro L. is an obligate air-breather, normally entering water only to drink or release eggs. The respiratory apparatus consists of paired lungs, formed by the evaginated lining of the branchiostegites, and 14 pairs of rather small gills (Semper, 1878; Harms, 1932). The lungs are ventilated by the anteriorly placed scaphognathites which draw air forwards through the lung. However, the efficiency of ventilation and the length of diffusion paths within the lung are not known. The epithelium and cuticle of the lining of the lung are thin and suited to a gas-exchange function (haemolymph–gas diffusion distance 0·5–1·2 μm) (Storch & Welsch, 1984). In contrast, the gills seem unsuited to gas exchange; their surface area is small and their structure, the nature of their attachment and their location

Key words: *Birgus*, haemocyanin, landcrabs, oxygen, carbon dioxide, gills, lungs.

in a pocket of the branchiostegite preclude their effective ventilation with air (Semper, 1878; Cameron, 1981*a*).

In the field, *Birgus* is slow-moving, and often remains immobile for long periods. The crabs may travel long distances in search of food, however, and on Christmas Island (Indian Ocean) appear to be semi-nomadic, moving through the rainforest in search of fruiting trees. They must also travel to the sea during the spawning season. Rapid locomotion is seen only on disturbance, but even then the crab soon backs into a crevice or climbs a tree pausing as soon as it reaches safety. On behavioural grounds it is apparent that the species does not sustain high levels of activity but is capable of sustained slow or intermittent activity. This is backed up by physiological evidence indicating that *Birgus* cannot sustain high levels of aerobic activity (Smatresk & Cameron, 1981). The crabs do, however, show ventilatory adaptations to exercise and can rapidly correct respiratory acidosis engendered by exercise.

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 role of the gills was investigated indirectly by studying the effects of gill ablation (Harms, 1932; Smatresk & Cameron, 1981). Ablation resulted in a small increase in ventilation rate and a reduced ability to cope with respiratory acidosis after exercise. At least in the short term, therefore, the gills appear to perform only a minor role in gas exchange and acid–base balance. Either the main site of gas exchange is normally the lung or the lung is capable of compensating for the gills.

In this study, the roles of the gills and lungs in gas exchange and acid–base balance of *Birgus* were examined by sampling pre- and postpulmonary and pre- and postbranchial haemolymph. This allowed more direct measurement of the contributions of each organ and avoided the problems of trauma and compensation by other organs following gill removal. Additionally, we investigated the effects of prolonged exercise on acid–base balance, production of lactic acid and its contribution to metabolic activity. *In vivo* data for oxygen content and partial pressure of the haemolymph have been combined with *in vitro* data for haemolymph (Morris *et al.* 1988) to determine the role of the pigment in oxygen carriage.

Materials and methods

Specimens of *Birgus latro* were collected from Christmas Island (Indian Ocean) and maintained at the University of New South Wales as described previously (Morris *et al.* 1988). For 24 h prior to sampling the animals were isolated without food and water in plastic fish-boxes at 25°C.

Measurement of haemolymph gas parameters

The partial pressures of oxygen and carbon dioxide (P_{O_2} and P_{CO_2}) in *Birgus* haemolymph samples were measured using the electrode/cuvette of a BMS 3

blood microsystem (Radiometer, Copenhagen, Denmark) thermostatted at 25 °C. Calibration was by humidified gas mixtures delivered by Wösthoff gas-mixing pumps (Type SA 18, Wösthoff, Bochum, FRG). P_{CO_2} was displayed on the expanded scale of a PHM 72 meter (Radiometer) and P_{O_2} on a Strathkelvin oxygen meter (Strathkelvin, Glasgow, UK). Haemolymph pH was measured using the G299a capillary electrode of the BMS 3 and displayed on a PHM 72 (Radiometer).

The oxygen content (C_{O_2}) of the haemolymph samples was measured by the method of Tucker (1967) according to Bridges *et al.* (1979) using 5 or 10 μl samples. The carbon dioxide content (C_{CO_2}) was determined by the method of Cameron (1971) employing 10 and 15 mmol l^{-1} $NaHCO_3$ standards.

[L-lactate] in the samples was determined using the Boehringer test kit (catalogue no. 139084, Boehringer Mannheim GmbH, Mannheim, FRG) and [urate] using the Sigma test kit (no. 685, Sigma Chemical Co., St Louis, USA). Concentrations of calcium and copper were determined with a Varian AA175 atomic absorption spectrophotometer as described previously (Sparkes & Greenaway, 1984).

Experimental protocol

Series A

In the first experimental treatment arterial and venous samples were taken from quiescent and exercised *Birgus latro* (400–500 g). Exercise was for a 30-min period in a humidified 25 °C constant-temperature room and was induced by repeatedly startling the animal into an escape response. Most specimens became refractory towards the end of this period. Samples were taken in 1 ml glass syringes the dead space of which had been previously filled with saline matching the osmotic pressure of the haemolymph and equilibrated with CO_2 (7·4 mmHg rest and 14·8 mmHg exercised). The samples were stored on ice for a maximum of 15 min and the final 30 % of the haemolymph was discarded.

Arterial haemolymph was sampled directly from the pericardial cavity *via* a small hole in the carapace dorsal and posterior to the heart and 2–3 mm to one side of the anterior–posterior axis. This hole was drilled at least 24 h prior to the experiment and did not penetrate the hypodermis. After sampling the hole was plugged with silicone grease.

Venous samples were withdrawn from the sinus at the base of the second walking leg which receives haemolymph returning from the leg and from the ventral sinus. It is possible, as with other decapods, that the sample does not represent fully mixed venous haemolymph. Each animal was used once only and the sampling period was <30 s. The number of animals in each group was never less than 10. In addition to measurement of P_{O_2}, P_{CO_2}, C_{O_2} and C_{CO_2} in arterial and venous haemolymph the concentrations of L-lactate, Ca, Cu and urate were also determined in this series. Differences between exercised and rested parameters were tested using the *t*-test and differences between arterial and venous data sets using a paired-sample *t*-test.

Series B

In this series the rested and exercised treatments were repeated but, in addition to arterial and venous haemolymph, samples of haemolymph returning from the lungs were taken from the pulmonary veins. The positions of these vessels were determined by examining casts of the vascular system of *Birgus* (C. A. Farrelly & P. Greenaway, unpublished data). In the crabs used, these veins were approximately 3 mm in diameter and were sampled *via* a second hole drilled in the dorsal carapace. Otherwise, sampling was as for the pericardial site and in general as described for series A. Large samples could be obtained from the exercised animals as the duration of the sampling period was less crucial. In rested animals only small samples could be obtained quickly and, as a consequence, the partial pressures of oxygen and carbon dioxide were not determined. Concentrations of Ca, Cu, L-lactate and urate were determined in arterial samples. Pulmonary – arterial and pulmonary – venous differences were tested using the *t*-test for paired samples.

Series C

In this series, afferent and efferent branchial haemolymph was sampled. The gills of *Birgus* are housed in a semiflexible pocket formed from the ventral margin of the carapace (Harms, 1932). The posterior margin is quite flexible and it was possible to move this aside to expose the last five gills (nos 10–14). Haemolymph leaving the gill lamellae passes into large efferent sinuses on the underside of the gill which in turn feed the branchiopericardial vessels running to the pericardial cavity. Efferent branchial haemolyph was taken from these sinuses using a 25 μl gas-tight Hamilton syringe with an exchangeable 26 gauge hypodermic needle with low dead space which had been prefilled with saline. Haemolymph was sampled directly from the efferent sinuses of gill 10 with a success rate >90 %. In the few cases where a sample could not be obtained within 2 min the animal was discarded for the purpose of the experiment. When samples of 12–20 μl were obtained 5 μl subsamples were used for the determination of C_{O_2} and C_{CO_2}. When sample sizes >20 μl were obtained 10 μl was used in each case. In the short period between sampling and analysis, mixing between the saline in the dead space and haemolymph was restricted to a narrow boundary layer contained within the residual blood in the syringe.

A sample of afferent branchial haemolymph was taken immediately after the efferent sample (within 10 s). This was drawn from the venous sinus supplying the branchial circulation of that limb. Large samples (250 μl) could be taken and this allowed the determination of pH in addition to C_{O_2} and C_{CO_2}. The [Cu] was not determined in series C.

Owing to the nature of the sampling method used, animals in series C could not be considered quiescent but nor were they exercised. This group are accordingly referred to as disturbed animals throughout this report. Differences between pre- and postbranchial samples were tested using the *t*-test for paired samples.

Definitions

Pa_{O_2}	Arterial oxygen partial pressure
Pv_{O_2}	Venous oxygen partial pressure
Pa_{CO_2}	Arterial carbon dioxide partial pressure
Pv_{CO_2}	Venous carbon dioxide partial pressure
C_{O_2}	Oxygen content
C_{CO_2}	Carbon dioxide content
Ca_{O_2}	Arterial oxygen content
Cv_{O_2}	Venous oxygen content
Ca_{CO_2}	Arterial carbon dioxide content
Cv_{CO_2}	Venous carbon dioxide content
pHa	Arterial pH
pHv	Venous pH
Prebranchial	afferent supply to gill 10
Postbranchial	efferent supply from gill 10
Pulmonary	efferent supply from the branchiostegal lung

Results

Respiratory gas and acid–base measurements

The measurements made in series A are summarized in Table 1. In resting *Birgus* there were significant arterial – venous (a–v) differences in the P_{O_2}, C_{O_2}, P_{CO_2} and C_{CO_2} of the circulating haemolymph. Exercise changed the absolute values of measured parameters but the a–v differences were maintained. There was a significant a–v difference in the pH of the haemolymph of exercised crabs. Interestingly, exercise resulted in a significant decrease in Pa_{O_2} but Ca_{O_2} was unaffected. Conversely, Cv_{O_2} decreased without a decrease in Pv_{O_2}. Pa_{CO_2} and Pv_{CO_2} increased significantly as a result of exercise, whereas Ca_{CO_2} and Cv_{CO_2} declined significantly. This change in the carbonic acid equilibrium was reflected by an exceptional decrease in haemolymph pH. Both pHa and pHv fell by approximately 0·7 pH units during the 30 min exercise period. A major part of this acidosis was metabolic in origin as the concentration of circulating lactate increased by more than 27 mmol l^{-1}. There was an increase in the mean [Cu] of circulating haemolymph from 2·63 to 3·24 mmol l^{-1} but variability among individuals meant that the difference was not significant. The concentration of urate in the haemolymph of resting crabs was 0·23 mmol l^{-1} in these animals and the small increase with exercise was not significant. An increase in [Ca] of approximately 1 mmol l^{-1} was observed in both arterial and venous haemolymph after 30 min of exercise. Using combined data for arterial and venous [Ca] this increase was significant ($0{\cdot}05 < P < 0{\cdot}02$).

In series B the respiratory and acid–base parameters of haemolymph from the pulmonary vein (with the exception of P_{CO_2}) approximated those of the pericardial rather than venous haemolymph (Fig. 1). As in series A, exercise dramatically

Table 1. *Haemolymph gas and acid–base parameters for arterial and venous haemolymph from rested and exercised* Birgus latro *(series A)*

	Rested ($N = 12$)		Exercised ($N = 10$)	
	Arterial	Venous	Arterial	Venous
P_{O_2} (mmHg)	43·8 ± 28·9	10·0 ± 1·8**	27·0 ± 7·2†	12 ± 4·3**
P_{CO_2} (mmHg)	7·10 ± 0·69	8·00 ± 0·82**	14·6 ± 3·57†	17·6 ± 4·35*†
C_{O_2} (mmol l^{-1})	1·10 ± 0·19	0·67 ± 0·17**	1·00 ± 0·18	0·31 ± 0·10**†
C_{CO_2} (mmol l^{-1})	13·6 ± 2·2	14·4 ± 2·2**	6·6 ± 2·1†	7·5 ± 2·4**†
pH	7·731 ± 0·047	7·711 ± 0·047	7·066 ± 0·140†	7·019 ± 0·169**†
[Ca] (mmol l^{-1})	12·13 ± 1·02	12·41 ± 1·41	13·04 ± 1·55	13·15 ± 1·39
[L-lactate] (mmol l^{-1}) (pericardial)	0·20 ± 0·20		27·33 ± 4·23†	
[Cu] (mmol l^{-1}) (pericardial)	2·63 ± 0·45		3·24 ± 0·43	
[Urate] (mmol l^{-1}) (pericardial)	0·23 ± 0·42		0·31 ± 0·41	

* a–v difference, $0·01 < P < 0·05$.
** a–v difference, $P < 0·01$.
† Resting – exercised difference, $P < 0·05$.
The animals were exercised for 30 min and all values are means ± S.D.

altered the absolute values of all measured parameters but the relationship between the three samples persisted with the changes in the afferent pulmonary haemolymph largely mirroring those in the arterial samples. Maximal P_{O_2} of the pulmonary haemolymph in an exercised animal was 100·3 mmHg.

In an attempt to understand the role of the lung in gas exchange, pulmonary – arterial and pulmonary – venous differences in pH and gas content were calculated and tested for significance (Table 2). Arterial haemolymph from rested crabs contained significantly less oxygen than haemolymph returning from the lung, but considerably more than the venous sample. This trend was even clearer in exercised animals (Table 2), indicating that significant oxygen loading occurred in the lung. The situation for CO_2 was more complex. In rested *Birgus* there was no significant decrease in C_{CO_2} during passage from the venous system through the lung but, nevertheless, arterial haemolymph contained significantly less CO_2 than venous haemolymph, indicating either that CO_2 was excreted elsewhere or that an increase in pH had occurred. The measured pH differences were significant, but not in a direction that would support the titration of HCO_3^-. In the exercised crabs there was no significant difference in the C_{CO_2} of arterial and pulmonary haemolymph. However, the P_{CO_2} of postpulmonary haemolymph was greater than that of pericardial haemolymph. Despite these data some CO_2 appeared to be lost at the lung as both partial pressure and content decreased, the latter significantly (Table 2). The small but significant concomitant increase in pH could, however, largely account for decreased C_{CO_2} at a constant P_{CO_2}. The levels of Ca, Cu and L-lactate in the haemolymph all increased significantly during exercise, but the

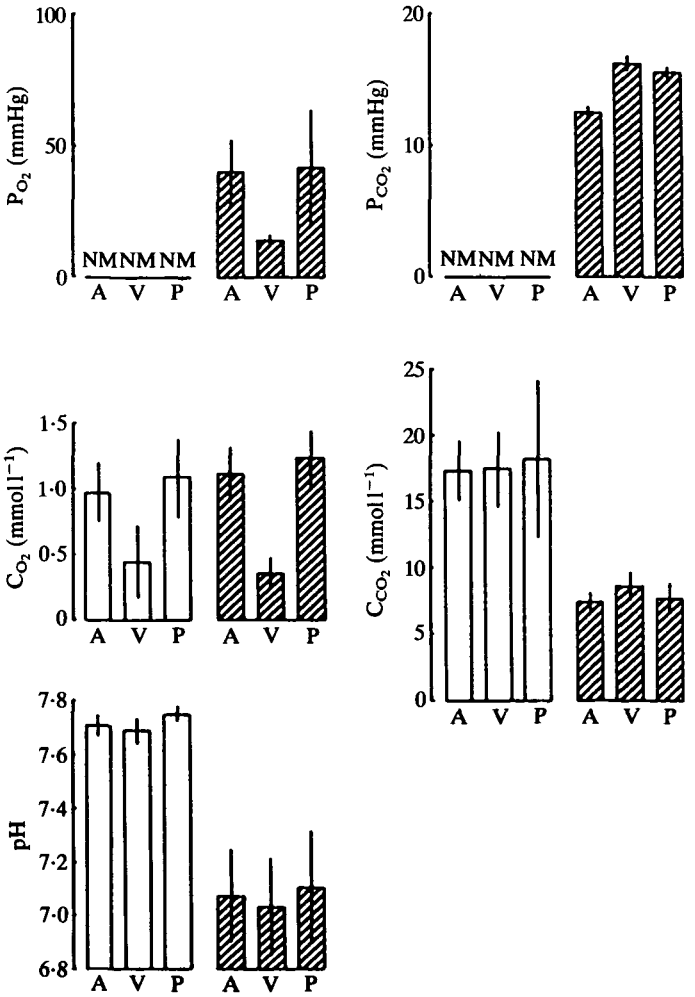

Fig. 1. Arterial (A), venous (V) and pulmonary (P) haemolymph gas and acid–base parameters in rested (open columns) and exercised (cross-hatched columns) *Birgus latro* at 25°C (series B). Values are means ± S.D. ($N = 10$). Sample size from rested *Birgus* was insufficient to allow the measurement of partial pressures. NM, not measured.

concentration of urate was variable among animals and no significant trend could be detected.

The results of the investigation of changes in haemolymph gases during passage through the branchial circuit (series C) are summarized in Table 3. There was no significant increase in the C_{O_2} of the haemolymph during passage through the gill, but there was a highly significant loss of CO_2 as the mean C_{CO_2} decreased by 2·30 mmol l^{-1}. The mean prebranchial pH (7·465) was lower than the venous pH of rested *Birgus*. Assuming that all haemolymph pH changes during gill passage were due to the loss of CO_2 it was possible, using a relationship determined *in vitro*

Table 2. *Mean values (±S.D. N = 10) for pulmonary – arterial and pulmonary – venous differences in haemolymph gas and acid–base parameters for rested and exercised* Birgus latro *(series B)*

	Rested		Exercised	
	p–a	p–v	p–a	p–v
P_{O_2} (mmHg)			2·7 ± 12·3	27·2 ± 21·2**
P_{CO_2} (mmHg)			2·99 ± 1·93**	−0·73 ± 2·71
C_{O_2} (mmol l^{-1})	0·11 ± 0·15*	0·64 ± 0·26**	0·12 ± 0·17*	0·88 ± 0·20**
C_{CO_2} (mmol l^{-1})	0·92 ± 1·12*	0·72 ± 1·94	0·23 ± 0·99	−0·80 ± 0·63**
pH	0·032 ± 0·047*	0·061 ± 0·045**	0·030 ± 0·059	0·071 ± 0·062**
[Ca] (mmol l^{-1})	12·4 ± 1·3		14·4 ± 1·2‡	
[L-lactate] (mmol l^{-1})	0·31 ± 0·28		22·98 ± 6·84‡	
[Cu] (mmol l^{-1})	2·66 ± 0·56		3·16 ± 0·50†	
[Urate] (mmol l^{-1})	0·14 ± 0·08		0·31 ± 0·59	

* Significant p–a or p–v difference, $0·10 > P > 0·05$.
** Significant p–a or p–v difference, $P < 0·01$.
† Significant rested – exercised difference, $0·10 > P > 0·05$.
‡ Significant rested – exercised difference, $P < 0·01$.
Sample size was insufficient to permit the measurement of P_{O_2} and P_{CO_2} of haemolymph from rested animals.

Table 3. *Prebranchial and postbranchial gas and acid–base parameters (mean ± S.D., N = 12) in the haemolymph of disturbed* Birgus latro *(series C)*

	Prebranchial	Postbranchial
C_{O_2} (mmol l^{-1})	0·23 ± 0·24	0·31 ± 0·21
C_{CO_2} (mmol l^{-1})	18·97 ± 3·42	16·67 ± 3·30**
pH	7·465 ± 0·140	(7·595)
P_{O_2} (mmHg)	(6·5)	(7·2)
P_{CO_2} (mmHg)	(8·9)	(5·7)

** Significant prebranchial – postbranchial difference, $P < 0·01$.
The postbranchial samples were taken directly from the efferent vessel of gill 10.
The values in parentheses were extrapolated from mean values using relationships determined for *Birgus* haemolymph *in vitro* (Morris *et al.* 1988).
For further details see text.

for $\Delta C_{CO_2}/\Delta pH$ (Morris *et al.* 1988), to extrapolate to a postbranchial pH. This value (7·595) was similarly lower than the pHa measured in resting *Birgus*. The extrapolated values for post- and prebranchial P_{CO_2} were determined in similar fashion by inserting *in vitro* values together with the determined C_{CO_2} into the Henderson–Hasselbalch equation. This allowed estimation of values where none were measured (see Table 3).

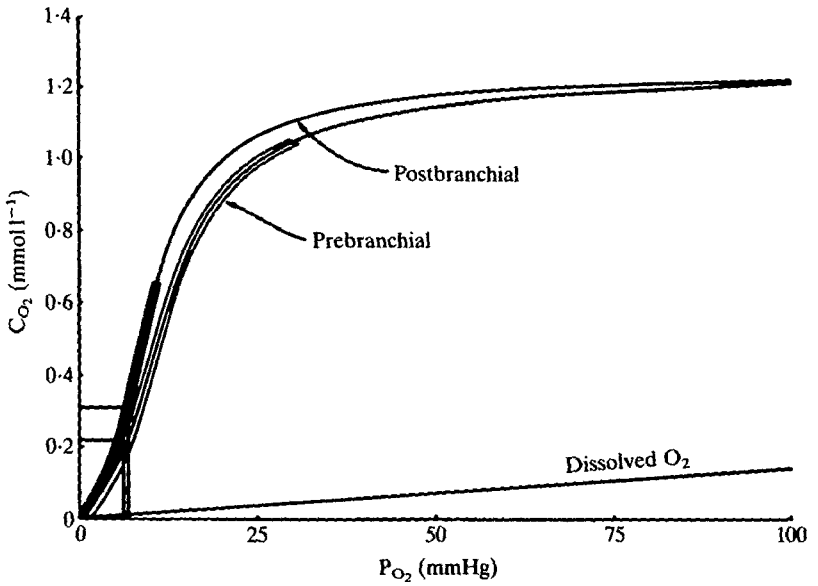

Fig. 2. Oxygen equilibrium curves calculated from *in vitro* data (Morris *et al.* 1988) for pH 7·465 and pH 7·595 at 25 °C. Together with these curves are plotted the measured oxygen content values of pre- and postbranchial haemolymph (series C). The filled part of the high-affinity curve represents the range of postbranchial values. The open area on the lower-affinity curve shows the range of prebranchial content values.

The mean pre- and postbranchial P_{O_2} values were derived as indicated in Fig. 2. Arterial P_{O_2} values approximated a mean value for C_{O_2} of 1·1 mmol l^{-1} and therefore the equilibrium curves were normalized to this value. The curves were constructed by abstracting the appropriate data for pre- and postbranchial pH from Morris *et al.* (1988). The mean C_{O_2} values were then plotted with these curves to supply the appropriate P_{O_2}. Also indicated in Fig. 2 is the range of C_{O_2} values measured and these data reflect that in 19 % of the animals there was a decrease in C_{O_2} during passage through gill 10.

The role of haemocyanin in oxygen transport

The concentration of Cu was used as a measure of haemocyanin concentration. Measurements of oxygen capacity in air-equilibrated haemolymph and [Cu] showed, however, that [Cu] overestimates Hc–O_2max by approximately 19 %. Therefore, haemocyanin–oxygen equilibria were calculated assuming that [haemocyanin] = 0·41[Cu] (Fig. 3). The arterial and venous data from rested crabs (Fig. 3A) and arterial, venous and pulmonary data from exercised crabs (Fig. 3B) were also described by equilibrium curves calculated from the previously determined *in vitro* relationship (Morris *et al.* 1988). The relative importance of dissolved compared with haemocyanin-bound oxygen was evaluated by normalizing 100 % saturation as 1·1 mmol l^{-1} and calculating the dissolved fraction over the appropriate P_{O_2} range (Fig. 3A,B).

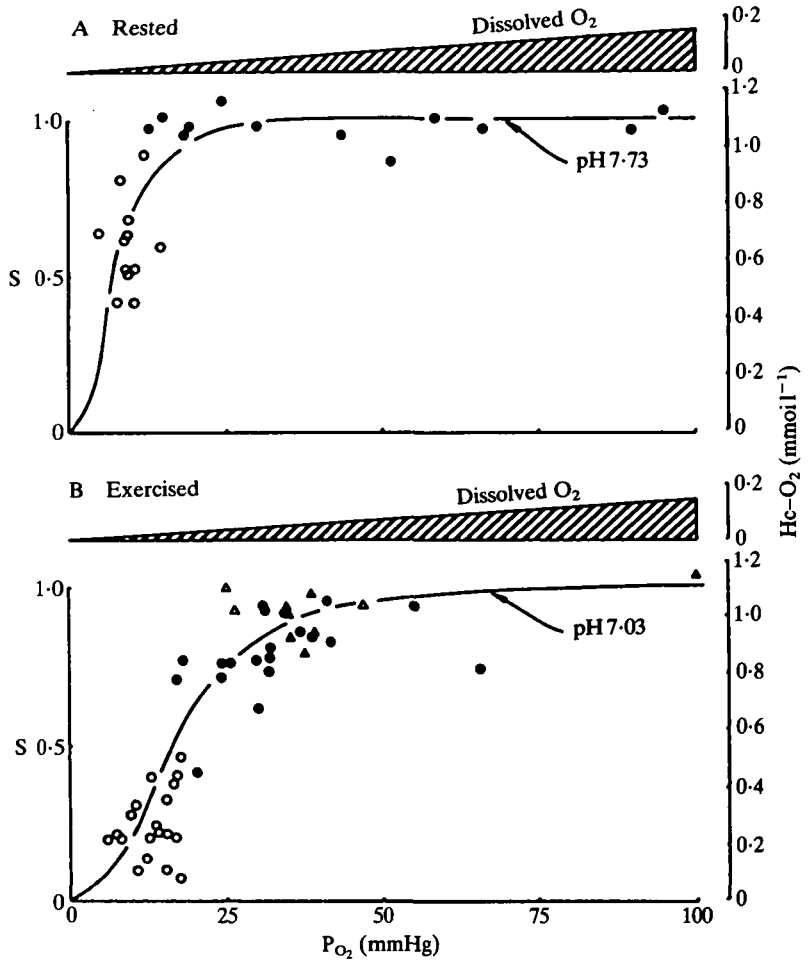

Fig. 3. Oxygen equilibrium curves derived from *in vitro* data for mean pH values measured in rested (A) and exercised (B) *Birgus* at 25°C. The data points for rested crabs are arterial (●) and venous (○) values derived from series A. Data for exercised crabs were taken from series A and B for arterial (●) and venous (○) values whereas pulmonary data (△) are from series B only. The measured C_{O_2} values of arterial, venous and pulmonary haemolymph were recalculated as saturation values assuming 81 % of the haemolymph Cu was incorporated in active haemocyanin. A normalized scale for Hc–O_2 is shown with Hc–O_2max = 1·1 mmol l^{-1}. The dissolved fraction must be added to this value to obtain the haemolymph O_2 content.

In resting *Birgus*, the haemocyanin of arterial, and therefore pulmonary, haemolymph remained saturated, and at the highest Pa_{O_2} recorded dissolved O_2 made up 10 % of the total haemolymph load. A fall from a mean arterial pressure of 44 mmHg to a venous tension of 10 mmHg reduced dissolved O_2 by 0·05 mmol l^{-1} and reduced haemocyanin saturation from 98 to 61 %. Thus a mean total of 0·46 mmol l^{-1} O_2 was extracted from the haemolymph by the tissues during each circuit of the haemolymph, with approximately 11 % of this coming

from the dissolved fraction. Haemolymph passing through the lung of resting *Birgus* was completely reoxygenated, often to levels above that of the arterial haemolymph, before returning to the heart (Fig. 1).

A different situation was observed in *Birgus* that had been exercised to near exhaustion (Fig. 3B). The large decrease in pH markedly right-shifted the equilibrium curve, reducing haemocyanin oxygen affinity. However, haemocyanin in the haemolymph leaving the lungs was still 93 % saturated, although in arterial haemolymph saturation fell to 79 %. Despite maintenance of Pv_{O_2} close to, or even greater than, pre-exercise levels, venous saturation fell to a mean value of 25 %. Under these conditions the a−v difference in the dissolved fraction was reduced to 0·02 mmol l^{-1}, whereas the Hc−O_2 difference averaged 0·70 mmol l^{-1} providing a total a−v difference of 0·72 mmol l^{-1}.

Using the Ca_{O_2}, Cv_{O_2} and pulmonary O_2 contents (0·92, 0·29 and 1·08 mmol l^{-1}, respectively) further analysis of lung and gill function was possible. Assuming that efferent pulmonary and branchial supplies were the only return to the heart, and bearing in mind that no branchial uptake of O_2 occurred, the dilution of pulmonary haemolymph by efferent branchial haemolymph could be determined on a percentage basis. Accordingly, it was calculated that 79 % of the return to the heart came from the pulmonary circuit and 21 % from the branchial circuit, a ratio of 3·8:1 in favour of the lung.

Anaerobiosis and acid–base balance

To analyse further the changes in acid–base balance that occurred in exercising *Birgus* (Fig. 4), mean values calculated from experimental series B and C were

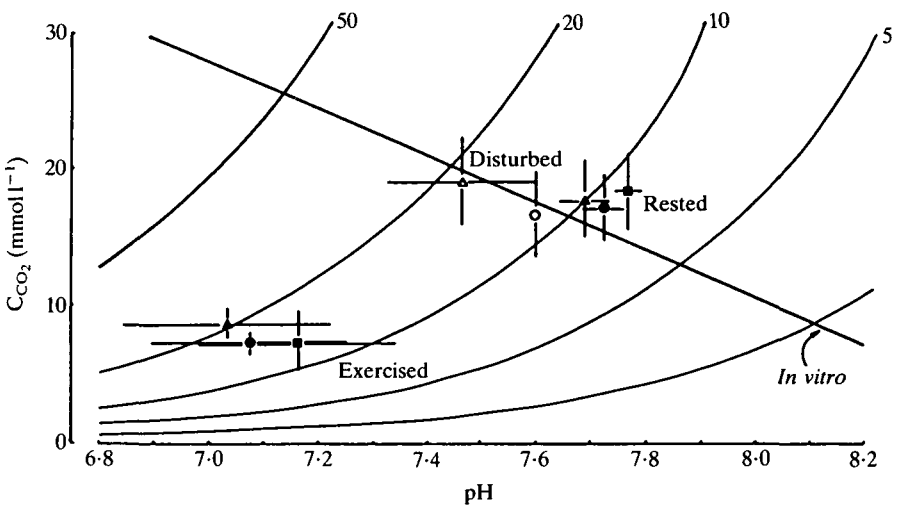

Fig. 4. pH/C_{CO_2} diagram for measured acid–base parameters at 25°C. Arterial (●), venous (▲) and pulmonary (■) haemolymph of rested and exercised *Birgus* (series B and C) were fitted. In addition the partially derived values for pre- (△) and postbranchial (○) haemolymph are also shown. Values are means ± S.D.

fitted to a C_{CO_2}/pH diagram. The similarity of arterial, venous and pulmonary parameters in the rested crabs was evident from the plotted mean values. A similar situation was seen for exercised crabs. Nevertheless, significant differences in C_{CO_2} and pH did exist within each of these groups (Table 2).

The large difference between the values for resting and exercised crabs was due to a complex change in acid–base balance of mixed respiratory and metabolic origin. The significant acidosis had been partially compensated by the end of 30 min of exercise. Despite the greater variation in values from exercised *Birgus*, the apparent titration down the nonbicarbonate buffer line from venous to pulmonary conditions was significant (Table 2). Thus, up to 0·8 mmol l^{-1} CO_2 was lost from the haemolymph of active crabs during each lung transit. In quiescent animals, the CO_2 lost *via* the lungs appeared negligible. Comparison of this value with the amount lost during gill transit (2·3 mmol l^{-1}) must take into consideration the greater relative flow through the lung calculated above. A value of 0·5 mmol l^{-1} CO_2 lost by all routes from the haemolymph of quiescent crabs per circuit was calculated accordingly. The available data for pre- and postbranchial haemolymph were fitted to the same diagram and indicated that the perturbations of acid–base balance resulting from disturbance could be accounted for by an increase in C_{CO_2} titrating pH along the nonbicarbonate buffer line.

The roles of lactate, H^+ and CO_2 generation in determining the acid–base balance of exercising *Birgus* were analysed in detail (Fig. 5). In this analysis, measured *in vivo* values were used to predict the expected pH; (*a*) assuming a change in P_{CO_2} alone (*b*) assuming a stoichiometric release of lactate and H^+ into the extracellular compartment and (*c*) taking into account changes in both measured [L-lactate] and P_{CO_2}. In Fig. 5 the difference *ac* is the depression in pH expected given the increase in L-lactate, *ab* is the actual change in pH and *bc* is the difference due to changes in P_{CO_2}. The predicted end-point position, B_3, was below the measured value which implies that the haemolymph was losing and/or gaining H^+ and HCO_3^- at differential rates, thus raising pH above that predicted. The observed [L-lactate] in the haemolymph could more than account for the very low pH in exercised animals assuming stoichiometric release of protons.

Discussion

Gas exchange

The lungs of *Birgus latro* were clearly effective in oxygen uptake as the haemocyanin in pulmonary haemolymph was saturated in both resting and exercised animals and high Pa_{O_2} levels were attained. In contrast, no significant contribution to oxygen uptake was discernible by gill 10. This situation obtained in the gills generally as pericardial haemolymph (mixed pulmonary and branchial) had a lower oxygen content than pulmonary haemolymph, indicating a dilution of oxygen-rich pulmonary haemolymph with low-oxygen branchial return.

Available evidence suggested that elimination of CO_2 was apportioned rather differently. Some loss of CO_2 across the lungs of *Birgus* must have occurred by

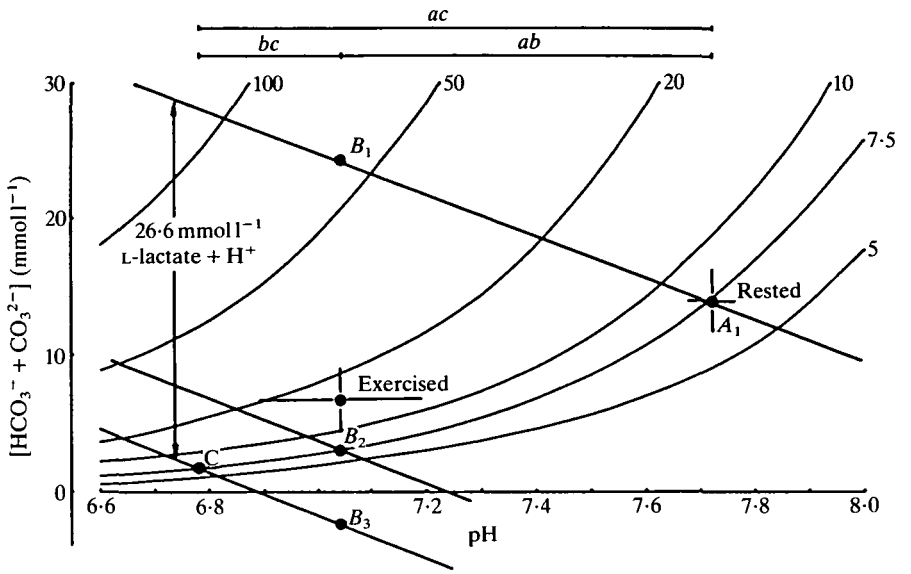

Fig. 5. The pH/[HCO_3^-] diagram for *Birgus* haemolymph at 25°C. P_{CO_2} units on isopleths are in mmHg. The buffer line is that determined *in vitro* (Morris *et al.* 1988). Actual data are combined mean values for venous and arterial haemolymph of rested and exercised *Birgus* (series A). This figure is used to determine what fraction of the total fall in pH (*ab*) was due to L-lactate (*ac*/*ab*) and what fractional pH change could be attributed to changes in P_{CO_2} (*bc*). A_1 is the measured resting value; B_1 is the observed fall in pH due solely to increased P_{CO_2}; B_2 is the fall in pH attributed to lactic acid alone; B_3 is the position expected, given the measured changes in L-lactate and P_{CO_2}; *C* is the pH expected following the addition of 26·6 mmol l^{-1} lactic acid in the absence of a P_{CO_2} change. (*ac*/*ab* = 1·38). For ease of reference the nomenclature of Wood *et al.* (1977) has been retained. For further details see text and Wood *et al.* (1977).

simple diffusion in all crabs, as the lungs are permeable to gases (viz high uptake of O_2) and a partial pressure gradient for CO_2 existed. Such loss was obviously negligible in resting animals. However, the higher P_{CO_2} levels in exercised animals and reported hyperventilation with exercise (Cameron & Mecklenburg, 1973; Smatresk & Cameron, 1981) may have enhanced diffusional loss enough to explain the measured loss (0·8 mmol l^{-1}) by this route following exercise. Carbonic anhydrase has been identified in the lipid (membrane-bound) fraction of lung epithelial cells and may have catalysed CO_2 formation from the bicarbonate pool in the haemolymph (S. Morris & P. Greenaway, unpublished data).

Significant diffusional loss of CO_2 across the gills by passive means was unlikely, given the measured P_{CO_2} gradient and the negligible inward movement of O_2. Carbonic anhydrase, however, was present in both lipid (membrane-bound) and cytoplasmic fractions of the gills of *Birgus* (S. Morris & P. Greenaway, unpublished data) and may have been responsible for the observed elimination of CO_2.

The lungs contained only membrane-bound enzyme at a lower activity (S. Morris & P. Greenaway, unpublished data).

Anaerobiosis does not produce CO_2 although the accompanying acidosis may transiently increase loss of CO_2 previously produced. High metabolic activity in *Birgus* will not, therefore, be accompanied by a proportionate increase in CO_2 output. Repayment of the oxygen debt incurred will require elimination of CO_2, but full recovery requires several hours (Smatresk & Cameron, 1981). Use of anaerobic metabolism during periods of high metabolic activity will minimize the rate of CO_2 elimination required and may be valuable in an animal where branchial gas exchange is demonstrably limiting.

The significance of gills and lungs in the elimination of CO_2 depends on haemolymph flow to these organs as well as the fractional removal achieved. When both these factors were considered it was apparent that the lungs and gills eliminated approximately equal amounts of CO_2 in exercised crabs (provided that there was no increase in fractional removal by the gills following exercise).

Cardiac output is related to the oxygen-carrying capacity of the haemolymph and, using available data (McMahon & Wilkens, 1983), the cardiac output of *Birgus* was estimated at $45\,ml\,kg^{-1}\,min^{-1}$. Using this value and our calculated value for removal of CO_2 from the haemolymph of resting *Birgus* ($0{\cdot}5\,mmol\,l^{-1}$), the rate of CO_2 output ($\dot{M}_{CO_2}$) was calculated to be $22{\cdot}5\,\mu mol\,kg^{-1}\,min^{-1}$. This compares favourably with the $\dot{M}_{CO_2}$ measured by Cameron & Mecklenburg (1973), $24\,\mu mol\,kg^{-1}\,min^{-1}$, and yields a value for the respiratory exchange ratio (R) of 0·94.

Acid–base balance

Exercise involved a very high level of anaerobic metabolism which resulted in a 100-fold increase in circulating levels of L-lactate to a mean level of $25\,mmol\,l^{-1}$ and in excess of $40\,mmol\,l^{-1}$ in one individual. These values are much higher than reported for other crustaceans and more than twice that found previously for *Birgus* following a 5-min period of exercise on a treadmill (Smatresk & Cameron, 1981). The leg muscles of *Birgus* possess a high activity of lactate dehydrogenase which can turn over up to 0·01 % of muscle mass min^{-1} as L-lactate (S. Morris & P. Greenaway, unpublished data).

The 30-min period of exercise caused an extremely large mixed acidosis of the haemolymph despite the relatively high buffering capacity of the haemolymph proteins. Measured levels of L-lactate more than accounted for this change, and it was evident that significant compensation occurred during the exercise period. In disturbed animals the observed acid–base changes were explicable as a respiratory acidosis.

Compensation for metabolic acidosis may have been achieved by several mechanisms. First, there was an increase in haemolymph [Ca] of $1–2\,mmol\,l^{-1}$ during exercise. Assuming that this was drawn from exoskeletal $CaCO_3$, the resultant formation of HCO_3^- would remove $2H^+$ per Ca^{2+} and this alone could account for the observed compensation of the acidosis excess. A second

mechanism of compensation involving branchial exchange of ions (H^+/Na^+ and HCO_3^-/Cl^-) is theoretically possible. Several species of landcrabs have been shown to release isosmotic urine into the branchial chambers where salt resorption occurs across the gills (Wolcott & Wolcott, 1982, 1984, 1985). Estimations of urinary salt output from data in the literature (Gross, 1964; Cameron, 1981*b*; Kormanik & Harris, 1981) suggest that a maximum of 22 mmol kg^{-1} day^{-1} of NaCl is available for ionic exchange. Substantial resorption of NaCl from the urine certainly occurs in the branchial chambers (P. Greenaway & S. Morris, unpublished data) and this would allow excretion of protons into the urine. Clearly this mechanism too could account for the compensation seen, but at present must be speculative.

A third possible mechanism of compensation is that differential efflux of H^+ and lactate or H^+ and HCO_3^- may occur from the metabolizing muscle tissue. Boutilier *et al.* (1986) have reported preferential release of H^+ from *Xenopus* muscle when the pH of the extracellular fluid was high, switching to preferential release of lactate as pH fell during exercise. From the evidence (Fig. 5), it is possible that the latter is occurring in exercised *Birgus* with retained H^+ being buffered intracellularly, and again this could explain much of the apparent compensation.

In a previous study of acid–base balance in *Birgus* (Smatresk & Cameron, 1981), an appreciable portion of the metabolic acidosis immediately after exercise could not be explained by the measured [L-lactate] and it was suggested that some other unidentified metabolite accounted for the remainder of the acidosis. In this study, protons accompanying L-lactate alone could account for all the observed acidosis and no other organic metabolites need to be considered. It is possible that differential release of H^+ and lactate from muscle cells, discussed above, may be responsible for the lack of stoichiometry in haemolymph H^+ and lactate loads observed in the previous study.

Role of haemocyanin

The oxygen content of arterial haemolymph of *Birgus* (1·1 mmol l^{-1}) is at the top of the range for crustaceans (McMahon & Burggren, 1988). Transport of oxygen in simple solution was always small (<0·2 mmol l^{-1}) and the high oxygen capacity was conferred by a high concentration of haemocyanin.

Utilization of circulating oxygen increased from 0·46 mmol l^{-1} (11 % dissolved) to 0·72 mmol l^{-1} (2·8 % dissolved) following exercise, an increase of 57 %, and oxyhaemocyanin was clearly the major carrier of oxygen in the haemolymph. Increased delivery in exercise was achieved by maintaining near saturation of arterial haemocyanin whilst the large (for a terrestrial crab) Bohr shift (Morris *et al.* 1988) depressed Cv_{O_2} (despite a small rise in Pv_{O_2} from 10 to 13 mmHg).

Although the *in vitro* Bohr shift was large between pH 8 and pH 7·3, below this it decreased sharply (Morris *et al.* 1988). This prevented extreme right-shifting of the equilibrium curve which would have compromised oxygenation at the lungs and thus reduced Ca_{O_2} and oxygen delivery. Increasing temperature decreased

oxygen affinity in *Birgus* (Morris *et al.* 1988) and it is clear from the *in vitro* and *in vivo* studies that temperatures above 25 °C in exercised crabs would compromise oxygen loading during exercise. In the resting state, however, a temperature increase reducing affinity could potentially increase oxygen delivery to the tissues. Thus the increased metabolic demand for oxygen with rising temperature could be satisfied in resting animals but would exacerbate problems of oxygen delivery during exercise.

The haemocyanin of *Birgus* was largely insensitive to [L-lactate] (Morris *et al.* 1988) and there may be several reasons for this. First, potentiation of haemocyanin by L-lactate would marginally increase arterial oxygen saturation but would necessitate very low Pv_{O_2} levels to release adequate oxygen to the tissues. Resultant reduction of the P_{O_2} gradient from haemolymph to cells would then restrict diffusional delivery of oxygen. Second, the ability of *Birgus* to increase ventilation of the lungs by >500 % (Smatresk & Cameron, 1981) would effectively maintain arterial saturation during exercise. Thus potentiation by lactate would have no real selective advantage and, indeed, could be disadvantageous. This may provide an explanation for the insensitivity to modulators seen in the respiratory pigments of air-breathing crabs generally. Modulation of the pigment was chiefly by pH and thus was a function of acidosis.

The Pv_{O_2} of exercised crabs (12–13 mmHg, Table 1; Fig. 1) was high considering the high level of anaerobic metabolism and oxygen debt, suggesting that oxygen supply to active tissues was diffusion-limited. Oxygen delivery per circuit was increased (57 %) during exercise and further increase would be enabled by the reported increase in heart rate with exercise (Smatresk & Cameron, 1981). Even so, delivery was totally inadequate for sustained activity. The crab clearly has a large anaerobic capacity for violent exercise (defensive leg flicking and rearing) but this is normally sustained for only a short period during which the animal seeks a safe refuge where the large oxygen debt can be repaid.

We should like to thank Caroline Farrelly for her help on numerous occasions throughout this investigation and also David Hair for technical assistance. SM and BRM thank Professor T. Dawson, Dr A. M. Beal and other members of the School of Biological Science, University of NSW for their assistance and hospitality. Financial support was provided by ARGS grant A18616299 to PG; a NATO overseas fellowship (NERC, UK) and an AHFMR research allowance to SM; and NSERC grants A5762, T7670 and IC-0265 to BRM.

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