J. exp. Biol. (1979), **80**, 271–285
With 3 figures
Printed in Great Britain

VENTILATION, OXYGEN UPTAKE AND HAEMOLYMPH OXYGEN TRANSPORT, FOLLOWING ENFORCED EXHAUSTING ACTIVITY IN THE DUNGENESS CRAB *CANCER MAGISTER*

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18 August 1978

SUMMARY

Scaphognathite and heart-pumping frequencies, ventilation volume, cardiac output, oxygen uptake and oxygen transport by haemolymph have been studied in unrestrained Dungeness crabs (*Cancer magister*) before, immediately after, and during recovery from 20 min of enforced exhausting activity. Exercise increased oxygen uptake 4-fold. This increase was achieved by more than 2-fold elevation of both ventilation volume and cardiac output and by greater participation of haemocyanin in oxygen delivery. The elevated ventilation volume resulted entirely from an increase in scaphognathite pumping frequency, while the rise in cardiac output resulted largely from increase in stroke volume. Prior to exercise haemocyanin accounts for less than 50% of the oxygen delivered to the tissues. Following exercise this increases to over 80%, the additional oxygen release being mediated by a depression of prebranchial oxygen tension and a substantial Bohr effect resulting from build up of lactate ion in the haemolymph and subsequent fall in pH. These changes allowed % oxygen extraction from branchial water to be maintained at 28% despite a 2-fold increase in ventilation volume, and allowed an increase in %. oxygen extraction by the tissues. Despite these changes oxygen supply fell below demand during exercise, and considerable anaerobic metabolism resulted, as evidenced by a 9-fold increase in haemolymph lactate concentration. The resulting oxygen debt required 8–24 h for repayment. Aerobic metabolic scope, and mechanisms of increasing oxygen uptake and transport in this crab are compared with those of a range of fish species.

INTRODUCTION

The physiology of exercise has been extensively studied in mammals, particularly man, and maximal oxygen uptake, aerobic and anaerobic capacities, and the metabolic pathways involved in exercise and recovery are relatively well understood. Less information is available on the lower vertebrates but the information that we have allows comparison of homoeothermic vertebrates with poikilothermic ones, and of aquatic and air-breathing types, revealing that it was the evolution of homoeothermy coupled with the ability to utilize aerial oxygen, rather than the adaptation of air breathing *per se*, which allowed the marked increase in aerobic capacity shown by birds and mammals over the lower vertebrates (Brett, 1972; Bennett, 1978). The

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reduced aerobic capacity of the lower vertebrates results in lower capacities for sustained work and greater reliance on anaerobic metabolism to satisfy intense metabolic demands (Bennett, 1978). Brett (1972) pointed out that our knowledge of the exercise capabilities of fishes is largely limited to active or very active species. The capabilities and mechanisms of more sluggish fishes may be very different (see Wood, McMahon & McDonald, 1977; Hughes & Johnston, 1978).

Comparison between lower vertebrate and invertebrate systems is severely limited as very little work has been carried out on the exercise capabilities of invertebrates, especially water breathers. Thus the present study was undertaken to determine the responses of a benthic, normally sluggish invertebrate to strenuous or exhausting exercise. *Cancer magister* is particularly suitable, in that the respiratory patterns (McDonald *et al.* 1977) and oxygen uptake levels (McDonald, 1977) of acclimated, resting, unrestrained animals are known. Determination of maximal rates of oxygen uptake thus provide a measure of aerobic capacity in this animal which may be used to compare the effectiveness of oxygen uptake through the chitin-covered lamellar gill of the crab with the non-chitinous lamellar gills of the fishes and also allow comparison of the oxygen transport capabilities of the crustacean open circulatory system with the closed system of vertebrates. A portion of this work has been presented previously in outline (McMahon *et al.* 1977).

MATERIALS AND METHODS

Nine *Cancer magister* of body mass ranging from 551 to 960 g (mean 850 ± 119 S.D.) were obtained from commercial suppliers or collected directly from the beaches of San Juan Island, Washington, U.S.A. Experiments were carried out at both the Friday Harbor Laboratories of the University of Washington and at the University of Calgary. At both locations crabs were maintained for at least 1 week prior to experimental use on a sandy substrate, in shallow tanks of approximately 25 cm water depth and 100 l capacity, and fed fish twice weekly. Tanks were supplied with flowing, natural sea water at 8 ± 1 °C and 27‰ salinity. The crabs were exercised (at 8 °C) in similar chambers. The exercise regime involved keeping the animals in near continual locomotor activity by repeated prodding over a 20 min period. Towards the end of this time all crabs were refractory to all but severe tactile stimulation.

The frequency of scaphognathite pumping (f_{sc}) was quantified from scaphognathite-induced rhythmic fluctuations in the pressure waveform recorded from both branchial cavities (method developed from Hughes, Knights & Scammel, 1969). The frequency of heart pumping (f_H) was obtained from fluctuations in impedance between two fine (0·13 mm o.d.) insulated stainless-steel wires implanted above the pericardial cavity on either side of the heart (Ansell, 1973). Justification and detailed description of the techniques were presented by McDonald *et al.* (1977).

Branchial water flow (ventilation volume, $\dot{V}_w$) was determined by a method similar to the direct method of Johansen, Lenfant & Mecklenburg (1970). At least 2 days prior to the experiments, the animals were fitted with a small, light, Butyrex plastic, mask which isolated the animals' exhalant apertures. The mask was sealed to the animal with rubber latex sheet (dam) and cyanoacrylate cement which prevented any leakage. Although the mask fitted closely to the animal to reduce its dead space, it did no

interfere with the scaphognathites nor with the movements of associated appendages. After an initial period of 8–48 h in which animals tried to dislodge the masks, the crabs became quiescent and showed respiratory and circulatory patterns similar to those exhibited by acclimated unmasked crabs (McDonald *et al.* 1977). An electromagnetic, cannulation type, flow probe (Biotronix 2100, 10 mm i.d.) was fitted to the mask through a small aperture in a latex rubber septum positioned on the anterior end of the mask. The fitting was leak-proof yet the probe could easily be fitted or removed without removing the mask or seriously disturbing the animal. *In situ*, the probe was oriented by design of the mask in the normal excurrent water stream, 1–2 cm from the exhalant openings. The large size of probe chosen offered no appreciable resistance (measured as pressure difference) to flow. The probe was connected to a Biotronix Model 610 electromagnetic flow meter, set to mean flow. The output of the flow meter was displayed, together with branchial pressure and heart impedance signals, on a rectilinear writing oscillograph (Gilson, M8PM). The flow system was calibrated by passage of equivalent known volumes of sea water per unit time through the probe. During recording from quiescent animals the regular occurrence of apnoeic periods (pauses) allowed a natural and non-disturbing means of determining flow zero. Immediately after exercise, and at other times when pausing behaviour patterns were not exhibited, flow zero could be determined by momentary finger occlusion of the probe lumen.

The oxygen tension of inhalant (= ambient) water (P_{I,O_2}) was measured using a sample of sea water taken from a catheter positioned near the inhalant (Milne-Edwards) openings. Oxygen tension of mixed exhalant water (P_{E,O_2}) was measured on a sample taken into a 2 ml glass syringe from a catheter positioned in the exhalant water stream immediately adjacent to the flow probe. Percentage extraction of oxygen from inhalant water (% Extr_w) and oxygen consumption ($\dot{M}_{O_2}$) were calculated from ventilation volume, and the difference between inhalant and exhalant tensions using equations (1) and (2) below. Right and left branchial cavity pressure and branchial water flow were monitored virtually continuously from 1 h prior to exercise until 4 h after it, and immediately prior to subsequent haemolymph samples. Sampling of inhalent and exhalent oxygen tensions, scaphognathite rates and ventilation volume occurred over 2–5 min intervals, for a 10 min period immediately preceding times of haemolymph sampling as detailed below.

Haemolymph samples were drawn 10–15 min prior to the exercise period and at 0–10 min, 30 min, 1, 2 and 4 h, and at varying intervals up to 24 h after exercise. Postbranchial (= arterial) samples were withdrawn anaerobically from the lateral pericardial cavity, into an ice-cold glass syringe, via a needle inserted through a hole previously drilled through a carapace posterior and lateral to the heart (McDonald, 1977; McMahon *et al.* 1978). The original hole did not penetrate the epidermis and was sealed with rubber sheet and cyanoacrylate glue to prevent blood loss after sampling. Postbranchial samples could be taken with very little disturbance to the animal. Prebranchial (venous) samples were taken by syringe and needle puncture of the arthrodial membrane over the infrabranchial sinus at the base of the 3rd or 4th walking limbs. Sites were protected with rubber as above. Prebranchial samples involved disturbance to the animals and consequently were taken on only five animals. A total of up to 0·8 ml of blood was taken per sample: each animal thus suffered a

maximum blood loss of 15 ml, or approximately 5% haemolymph volume, over a 24 h period. Each sample was analysed for oxygen tension (P_{O_2}), oxygen content (C_{O_2}), pH, and lactate ion concentration.

Oxygen tensions of sea water and haemolymph were measured using Radiometer oxygen microelectrodes, thermostatted to the experimental temperature and calibrated with either nitrogen equilibrated or air equilibrated sea water. Oxygen content was determined on an 80 μl sample injected into a Lex-O_2-Con oxygen content analyser (Lexington Instruments) using a higher than normal flow rate of gas (80 ml/min of 2% H_2+1% CO in N_2) to ensure good elution of oxygen. The accuracy of the analyser (±5% at these low oxygen contents) was checked regularly with samples of air-equilibrated distilled water. Concentrations of lactate ion in the haemolymph were analysed enzymically using Sigma reagents (Sigma bulletin No. 286UV).

Oxygen equilibrium curves were generated from 5 ml samples of haemolymph removed, after recovery from exercise, from individual crabs. Haemolymph was centrifuged to remove clotted protein to equilibration with humidified gas mixtures in an intermittently rotating tonometer maintained at experimental temperature. Curves were generated at each of 4 CO_2 tensions (0·7, 1·5, 4·0 and 6·2 torr P_{CO_2}). At each CO_2 tension haemolymph oxygen content was measured (Lex-O_2-Con) in haemolymph equilibrated over a range of oxygen tensions. Gas mixtures were produced using Wösthoff gas mixing pumps. Sets of equilibrium curves were generated for most animals used in this study. A considerable range of oxygen capacities occurred and thus a representative set of curves determined for a haemocyanin sample of average content ($C^{max}_{Hcy\,O_2}$ = 0·33 mmol.l^{-1} is presented in this account.

Calculations

Percentage oxygen extraction (% $Extr_w$) from branchial water flow ($\dot{V}_w$) was calculated from:

$$Extr_w\,\% = \frac{P_{I,O_2} - P_{E,O_2}}{P_{I,O_2}} \times 100, \tag{1}$$

where P_{I,O_2} = oxygen tension of inhalent water and P_{E,O_2} is the oxygen tension of mixed exhalant water.

Oxygen uptake ($\dot{M}_{O_2}$) from branchial water flow ($\dot{V}_w$) was calculated from:

$$\dot{M}_{O_2} = (P_{I,O_2} - P_{E,O_2})\,\alpha_{wO_2} \cdot \dot{V}_w, \tag{2}$$

$\dot{M}_{O_2}$ is in mmol O_2 kg^{-1}.min^{-1}, where $\dot{V}_w$ is in ml. kg^{-1}.min^{-1} and α_{wO_2}, the solubility coefficient for oxygen in water at appropriate temperature and salinity (Carpenter, 1966), is in mmol O_2.ml H_2O^{-1}.torr^{-1}.

Percent oxygen extraction from haemolymph by tissues ($Extr_b$ %) was calculated from:

$$Extr_b\,\% = \frac{C_{a,O_2} - C_{v,O_2}}{C_{a,O_2}} \times 100, \tag{3}$$

where C_{a,O_2} and C_{v,O_2} are oxygen content of postbranchial and prebranchial haemolymph respectively.

Cardiac output ($\dot{V}_b$) was caculated using the Fick principle

$$\dot{V}_b = \frac{\dot{M}_{O_2}}{C_{a,O_2} - C_{v,O_2}} \times 1000, \tag{4}$$

$\dot{V}_b$ is in ml. $kg^{-1}.min^{-1}$, where $\dot{M}_{O_2}$ is in mmol. $kg^{-1}.min^{-1}$ and C_{a,O_2} and C_{v,O_2} are in mmol $O_2.l^{-1}$.

Stroke volume (V_s, H), in ml. $kg^{-1}.beat^{-1}$, was calculated from

$$V_s, H = \frac{\dot{V}_b}{f_H}, \tag{5}$$

where f_H is frequency of heart pumping in beats.min^{-1} and $\dot{V}_b$ has the units above.

The log mean gradient for oxygen tension across the gills (ΔP_{O_2}, Hughes & Morgan, 1973) was calculated from

$$\Delta P_{O_2} = \frac{(P_{I,O_2} - P_{a,O_2}) - (P_{E,O_2} - P_{v,O_2})}{\log_n [(P_{I,O_2} - P_{a,_2})/(P_{E,O_2} - P_{v,_2})]} \tag{6}$$

and the transfer factor for oxygen (ΔT_{O_2}) was calculated using the oxygen equilibrium curves for *C. magister* haemolymph at pre-exercise and post-exercise pH generated as above, and the modified Bohr integration method proposed by Piiper & Baumgarten-Schuman (1968). Oxygen consumption was divided into 10 steps and ΔT_{O_2} calculated for each step,

$$\Delta T_{O_2} = \frac{\dot{M}_{O_2}.n^{-1}}{\Delta P}, \tag{7}$$

and the total value T_{O_2} obtained by summation.

RESULTS

Mean frequencies of heart and scaphognathite beat, branchial water flow, percentage oxygen extraction, and oxygen uptake recorded from unrestrained *Cancer magister* prior to, and immediately following, 20 min of enforced exercise are presented in Fig. 1 and Table 1. The values prior to exercise are representative of animals acclimated to their experimental chambers; quiescent, but exposed to slight disturbance caused by sampling techniques. The values recorded are typical of animals exhibiting continuous beating of both scaphognathites (bilateral pumping; McDonald, 1977; McDonald, McMahon & Wood, 1977).

Observation revealed that the 20 min exercise period chosen involved severe (possibly maximal) energy expenditure from these normally sluggish animals. Initially the animals responded quickly to even slight tactile stimulus, but within 20 min had become refractory to all but the most severe stimulation and responded very slowly.

Exercise was accompanied by a marked increase in the activity of the scaphognathite pump. In nine animals mean scaphognathite beat frequency had increased from 75 to 150 beats/min and this was associated with an increase in branchial ventilation volume from 380 to 810 ml. $kg^{-1}.min^{-1}$ (Table 1, Fig. 1). Despite the marked increase in branchial water flow, there was no significant change in the percentage oxygen extracted from the branchial water, and consequently oxygen uptake varied

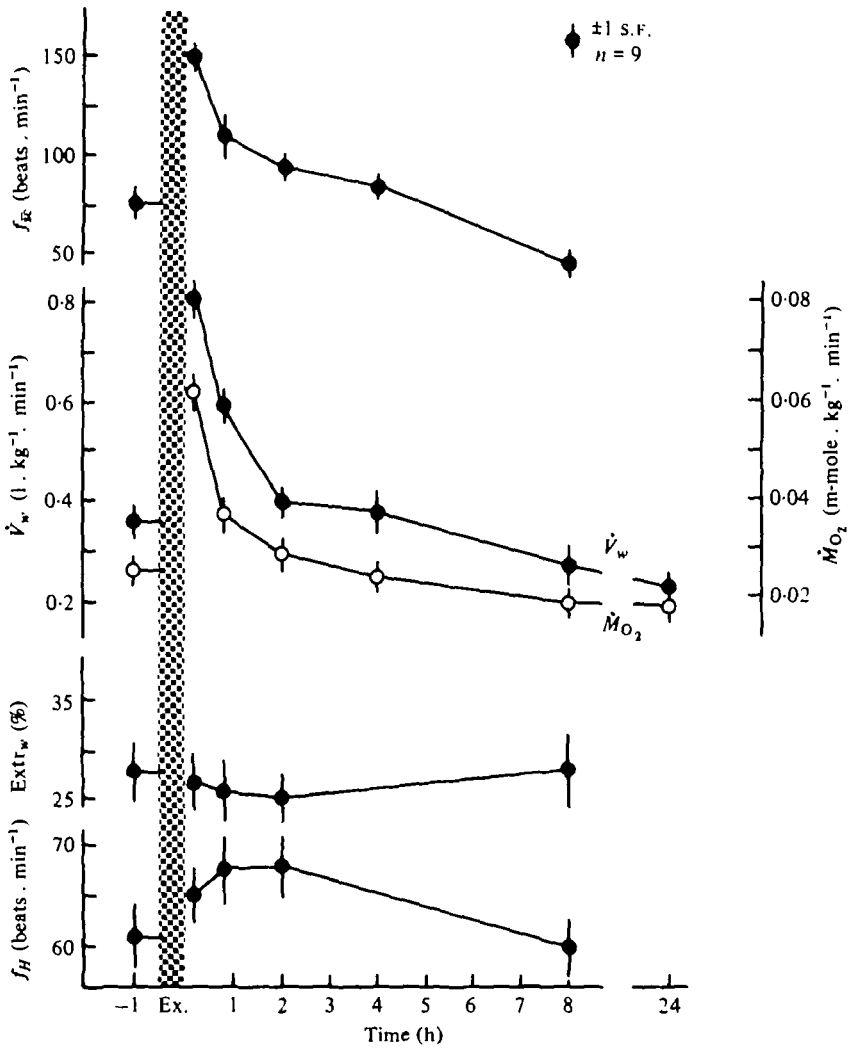

Fig. 1. Changes in the pumping frequencies of scaphognathites (f_{sc}) and heart (f_H) systems and in branchial water flow ($\dot{V}_w$), oxygen uptake ($\dot{M}_{O_2}$) and % oxygen extraction from branchial water flow (% Extr$_w$) in response to 20 min of enforced activity.

proportionally with branchial ventilation throughout these experiments. During recovery from exercise, oxygen consumption fell rapidly for the first 2 h, but then declined more slowly, reaching stable levels only after 8–24 h. This extended period of enhanced oxygen consumption strongly suggests the repayment of an 'oxygen debt' incurred during the exercise period. Haemolymph lactate levels increased 9-fold during the exercise period (Table 1) confirming that considerable anaerobic metabolism had occurred.

Heart rate also increased as a result of exercise, but the increases were small and variable (1–20%, mean 18%). Although small, the increase persisted for 2 h after exercise before declining to pre-exercise levels within 8 h. Heart and scaphognathite rates could be recorded during the exercise period in only two animals. These showed

Table 1. *Overall mean values* (± S.E.) *of respiratory and circulatory parameters in nine* Cancer magister *prior to and immediately after exercise*

	Before	After	Ratio
$f_{\bar{\omega}}$ †	74 ± 8	151 ± 5	2·04**
$\dot{V}_w$ (ml. kg^{-1}.min^{-1})	356 ± 25	810 ± 40	2·3**
$\dot{M}_{O_2}$ (nmol. kg^{-1}.min^{-1})	0·0246 ± 0·006	0·0609 ± 0·019	2·3**
$\dot{V}_w/\dot{M}_{O_2}$ (l. mmol^{-1})	14·2	13·3	0·94‡
Extr$_w$ (%)	28 ± 6	26 ± 6	0·93 N.S.
P_{I,O_2} (torr)	141 ± 2	141 ± 2	† N.S.
P_{E,O_2} (torr)	97 ± 3	104 ± 5	1·12 N.S.
P_{a,O_2} (torr)	75 ± 5	45 ± 8	0·6**
$P_{\bar{v},O_2}$ (torr)	15 ± 5	10 ± 5 (5)	0·67 N.S.
ΔP_{O_2}	74	95	1·38‡
T_{O_2}	0·00032	0·00081	2·5‡
S_{a,O_2} (%)	99	94	0·95‡
$S_{\bar{v},O_2}$ (%)	55	15	0·27‡
Tissue O_2 transported by HCy (%)	42	79	1·88‡
C_{a,O_2} (mmol O_2.l^{-1})	0·482	0·42 (5)	0·88 N.S.
$C_{\bar{v},O_2}$ (mmol O_2.l^{-1})	0·214	0·06 (5)	0·28**
Extr$_b$ (%)	56	86	1·54**
$\dot{V}_b$ (ml. kg^{-1}.min^{-1})	72 ± 25	185 ± 77	2·3**
$\dot{V}_b/\dot{M}_{O_2}$ (l. mmol^{-1})	3·74	2·79	0·75‡
$\dot{V}_w/\dot{V}_b$	3·87	4·76	1·23‡
f_{heart} (beat.min^{-1})	59 ± 2	69 ± 4	1·17*
S_v (ml. beat^{-1})	1·56	2·46	1·58**
pH_a	7·902	7·512	‡
p$H_{\bar{v}}$	7·895	7·493	‡
Lactate concentration (mmol. l^{-1})	1·0 ± 0·2	9·0 ± 1·2	8**

N.S., *Not significantly different.*
* Significantly different, $P = 0{\cdot}05$, by paired t test.
** Significantly different, $P = 0{\cdot}01$.
† $f_{\bar{\omega}}$, *the mean forward pumping rate for both scaphognathites, i.e.* $(R+L)/2$.
‡ Significance not determined.

increases of similar magnitude to the levels recorded immediately after exercise (Table 1) but these levels were achieved within 5 min, suggesting that they represent maximal levels attainable under these circumstances.

The pre-exercise levels of ventilation and oxygen extraction were sufficient to maintain mean postbranchial and prebranchial haemolymph oxygen tensions at 75 ± 5 S.E. torr and 15 ± 5 S.E. torr respectively. Equivalent mean oxygen contents were 0·48 and 0·22 mmol O_2.l^{-1} (Table 1, Fig. 2). Following the exercise period, oxygen tensions had decreased to 44 ± 8 S.E. in postbranchial and to 10 ± S.E. torr in prebranchial haemolymph. Despite the marked fall in oxygen tension, oxygen content of postbranchial haemolymph decreased only slightly to 0·42 mmol O_2.l^{-1}. Prebranchial oxygen content, however, decreased markedly to 0·07 mmol O_2.l^{-1} (Table 1, Fig. 2). Due to marked decrease in both postbranchial and prebranchial oxygen tensions occurring following exercise, together with little change in the P_{O_2} of inspired and expired water, the average mean oxygen pressure gradient across the gills (ΔP_{O_2}: Hughes & Morgan, 1973) increased from 74 to 95 torr.

To ascertain the role of haemocyanin in oxygen transport prior to and after exercise, mean oxygen pressures determined *in vivo* (Table 1) were plotted on a representative family of oxygen equilibrium curves for this species, determined *in vitro* over a physiological range of pH (McDonald, 1977; B. R. McMahon *et al.* in preparation).

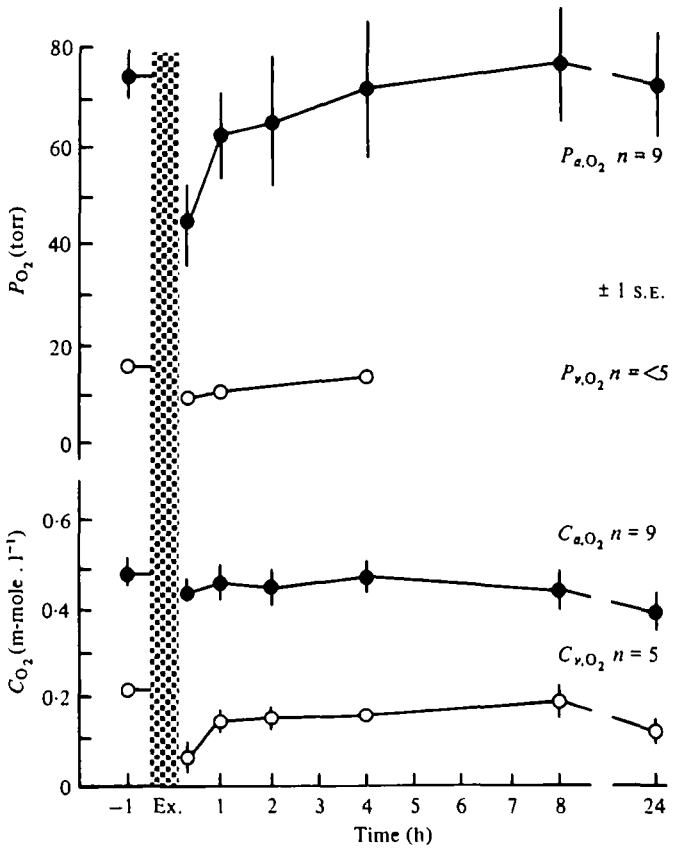

Fig. 2. Changes in oxygen tension and in oxygen content of postbranchial (P_{a,O_2}, C_{a,O_2}) and prebranchial (P_{v,O_2}, C_{v,O_2}) haemolymph in response to 20 min of enforced activity.

Prior to exercise the pH of postbranchial haemolymph was 7·90 and that of prebranchial haemolymph slightly lower at 7·89, corresponding approximately to a P_{CO_2} of 1·5–2 torr. At similar pH, Fig. 3 indicates that the haemocyanin of postbranchial haemolymph would be virtually 100% saturated, while that of prebranchial haemolymph might reach 60% saturation. Under these conditions only 40% of the oxygen delivered to the tissues would be supplied by haemocyanin: the remainder from physical solution in the haemolymph.

During exercise the oxygen content of postbranchial haemolymph decreased only slightly (Table 1). This decrease was largely in the dissolved oxygen fraction, and postbranchial haemocyanin remained virtually fully oxygen saturated. Oxygen content of prebranchial haemolymph, however, had decreased markedly following exercise. The decrease in oxygen pressure was only 5 torr, and thus little change occurred in the dissolved oxygen fraction, but the percentage oxygen bound to haemocyanin decreased from 55% to 15%. Only a fraction of this depletion would result from decrease of P_{O_2} at constant pH, but prebranchial pH falls to a mean of 7·493 following exercise (Table 1), and Fig. 3 demonstrates the additional release of oxygen resulting from the Bohr effect. Immediately following exercise, more oxygen is delivered to the tissues and a substantially greater fraction – over 80% – is now released from haemocyanin.

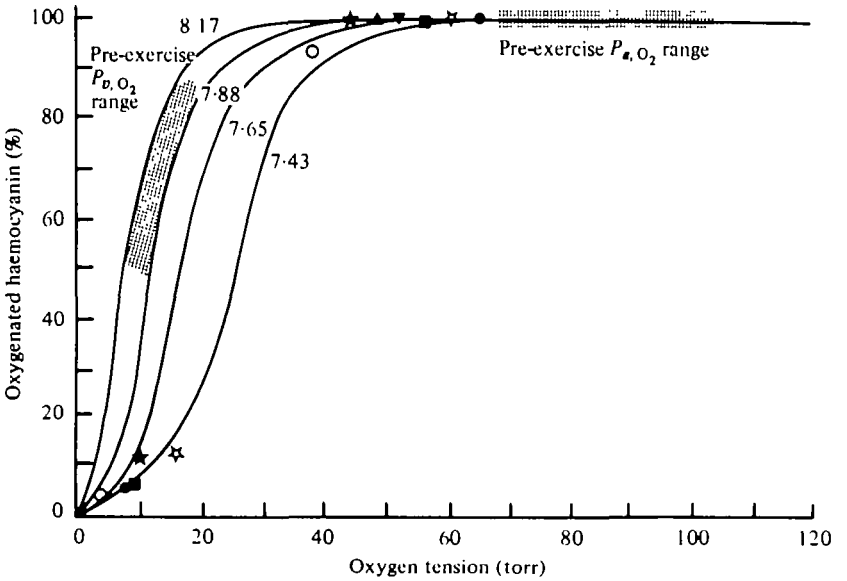

Fig. 3. A comparison of oxygen tensions and % saturation of haemocyanin in pre- and post-branchial haemolymph under conditions prevailing before and just after exercise. Oxygen equilibrium curves were determined *in vitro* on haemolymph from a single animal of average oxygen capacity equilibrated with gas mixtures containing either 0·7, 1·6, 4·0 or 6·2 torr P_{CO_2}. pH given was determined on deoxygenated serum equilibrated at each P_{CO_2}. Symbols represent levels of individual animals determined just after exercise. Values for P_{a,O_2} and P_{v,O_2} before exercise are presented as a range to avoid confusion. Means are presented in Table 1.

Cardiac outputs of individual animals before and after exercise ($\dot{V}_b$: Table 1) were calculated, using the Fick principle, from measured postbranchial and prebranchial oxygen contents and oxygen consumption. Although these estimates were somewhat variable between individual animals, nonetheless mean cardiac output had more than doubled (increase significant at the 1% level) following exercise. As the frequency of heart pumping increased only 18%, the majority of this change resulted from an increase in stroke volume from 1·6 ml. kg^{-1}. $beat^{-1}$ prior to exercise to 2·5 ml. kg^{-1}. $beat^{-1}$ immediately after.

Mean prebranchial and postbranchial oxygen contents were also used to estimate the efficiency of oxygen extraction by the tissues ($Extr_b$%: Table 1). Unlike the efficiency of oxygen extraction by the gills ($Extr_w$%: Table 1) which remained unchanged following exercise, $Extr_b$% increased sharply. Thus, increased oxygen supply to the tissues after exercise occurs both by increased haemolymph supply and by increased oxygen extraction.

Fig. 2 outlines the rates of recovery of haemolymph oxygen levels following exercise. As described for scaphognathite pumping and related parameters above, initial recovery is relatively rapid, with all values reaching levels not significantly different from those recorded before exercise within 1–2 h. All values, however, continue to change slowly after this, and do not stabilize fully for 8–24 h.

DISCUSSION

The levels of exertion achieved by crabs in the present study were assessed by measurement of oxygen consumption immediately after exhausting activity and are therefore difficult to compare precisely with studies on other animals moving at controlled speed. While the values obtained are not necessarily equal to 'maximal oxygen consumption at burst activity' or 'active metabolic rate' as determined previously for fish species, Brett & Blackburn (1978) considered that the method we used provides a reasonable estimate of active metabolic rate in dogfish. The extent to which the normally sluggish *Cancer magister* exhibits such protracted strenuous activity under natural conditions is not known, but the experiments provide information on the maximum aerobic metabolic scope for activity, this term being preferred to the less accurate 'scope for activity' (Bennett 1972, 1978) and on the respiratory and circulatory adjustments involved in satisfying this increased oxygen demand.

During the exercise period *Cancer magister* were able to elevate oxygen uptake a mean 2·4-fold over pre-exercise levels. Mean scaphognathite pumping frequency, branchial water flow and oxygen consumption all reached peak levels within 5 minutes of exercise, and these probably represent the maximal levels attainable in this species at this temperature. Thus, maximal oxygen uptake for *Cancer magister* at 8 °C may equal 0·061 mmol.kg^{-1}.min^{-1}. This value is from 2- to 5-fold lower than maximal uptake values for five fish species at similar temperature given by Brett (1972) but these data are all taken from fishes which are more active in their natural habitat than is *Cancer magister*. Few data are available for slowly moving fishes, but it appears that such fish have similar metabolic rates to *C. magister*: Brett & Blackburn (1978) estimated active metabolic rate in a normally slow-moving elasmobranch, *Squalus acanthias* as 0·052 mmol O_2.kg^{-1}.min^{-1} at 10 °C and Poulsen (1963) reports active metabolic rates ranging from 0·047 to 0·120 mmol.kg^{-1}.min^{-1} for a range of slow-moving, cave-adapted amblyopsid fishes at 13 ± 2 °C.

The lower levels of oxygen consumption exhibited by animals in the present study prior to activity (0·025 mmol.kg^{-1}.min^{-1}) are equivalent to those of a range of fish species at equivalent temperature (Brett, 1972) but do not accurately represent standard metabolism of this crab. Our animals were not active in the locomotor sense and were well acclimated to their experimental conditions, but they were masked and subject to intermittent sampling procedures. These necessarily involved a small degree of disturbance, altered respiratory and circulatory patterns and slightly increased oxygen consumption. McDonald (1977) and McDonald *et al.* (1977) demonstrated lower levels of scaphognathite activity in quiescent undisturbed crabs, involving extended periods of unilateral scaphognathite pumping, reduced branchial ventilation volume and lower oxygen uptake (0·016 mmol.kg^{-1}.min^{-1}). These values more accurately represent standard metabolism and allow estimation of the aerobic metabolic scope (Bennett, 1978) which for *Cancer magister* at 8 °C is 0·045 mmol.kg^{-1}.min^{-1} (or a 4-fold increase) a value again much lower than that determined for more active fish such as sockeye salmon (0·54 mmol.kg^{-1}.min^{-1}) or even goldfish (0·13 mmol.kg^{-1}.min^{-1}) (Brett, 1972). However, Poulson (1963) reports aerobic scopes ranging from 0·039 to 0·1 mmol.kg^{-1}.min^{-1} for sluggish cave-adapted teleosts, and Brett & Blackburn (1978) estimate a value of only 0·036 mmol.kg^{-1}.min^{-1}, or a 3-fold

increase for the slow-moving elasmobranch *Squalus acanthias*. No comparative data are known for aquatic crabs, but in the largely terrestrial decapod *Cardisoma quanhumi* average maximal oxygen uptake values of 0·12 mmol.kg^{-1}.min^{-1}, and average aerobic metabolic scope of 0·097 mmol.kg^{-1}.min^{-1} were recorded by Herreid, Lee & Shah (1978) for animals exercised on a treadmill. However, the much higher experimental temperature and air breathing habit of this species do not allow true quantitative comparison between the two crabs. A somewhat increased aerobic metabolic scope might be expected from a terrestrial crab due to the lower convection requirement associated with air breathing.

In *Cancer magister* several mechanisms are involved in the increased oxygen uptake observed. Ventilation volume increases from 2- to 4-fold over the basal levels, thus increasing oxygen supply to the gills. As the scaphognathite of this crab operates as a fixed volume pump (McDonald, 1977), this increase in entirely dependent upon increased scaphognathite frequency. Increase in $\dot{V}_E$ of similar magnitude in exercising dogfish (Piiper, Meyer, Worth & Willmer, 1977), and of greater magnitude in more strenuously exercised trout (Stevens & Randall, 1967; Kiceniuk & Jones, 1977) were both achieved largely by increase in stroke volume of the respiratory pump rather than by increase of respiratory rate. The capability of many exercising fishes to utilize ram ventilation during swimming may partially explain this relationship.

Increased perfusion of the gills with haemolymph also facilitates increased oxygen uptake resulting from exercise in *Cancer magister*. Here, as in both rainbow trout (Stevens & Randall, 1967; Kiceniuk & Jones, 1977) and the dogfish, *Scyliorhinus stellaris* (Piiper *et al.* 1977), increase occurs largely by elevation of stroke volume rather than pumping frequency. The exact mechanisms involved in the increase of stroke volume are not known in any crustacean, but several authors (reviewed by Maynard, 1960) describe increase in both rate and amplitude of heart beat with increase in haemolymph pressure. Perhaps an increase in pressure or in 'venous return' caused by muscle pumping during activity stimulates cardiac output by a stretch mechanism analogous to the well known Frank–Starling mechanism observed in vertebrate closed systems (Starling, 1918; Johansen, 1962; Randall 1970*a*). The projected increase in stroke volumes (1·44–2·6 ml. kg^{-1} body mass) is well within the range reported by McMahon & Wilkens (1977) for the related *Cancer productus* during its normal periodic pumping patterns, is similar to the figure calculated by Belman (1975) for the lobster *Panulirus*, and is also within the ranges calculated by deFur & Mangum (1978) for a range of crustacean species. The latter authors also report good agreement between cardiac outputs calculated (as in the present study) by the Fick principle and by anatomical estimates of heart capacity.

Oxygen uptake is also enhanced by changes in the oxygen binding characteristics of haemocyanin induced during the exercise period. Under conditions prevailing in the haemolymph prior to exercise the prebranchial to postbranchial oxygen tension gradient is large and prebranchial oxygen tension sufficiently high to allow more than 50% haemocyanin oxygen saturation. Under these conditions haemocyanin plays a relatively minor role, accounting for less than 40% of the oxygen carried to the tissues: the major portion being delivered from physical solution. A similar situation has been described for *Homarus americanus* under similar conditions (McMahon & Wilkens, 1975). *Cancer magister* haemocyanin, however, seems particularly well suited to

function under exercise conditions. Haemolymph oxygen tensions fall following strenuous activity in crabs (Johansen *et al.* 1970; Mangum & Weiland, 1975). In the present study an approximately 40% reduction had occurred in both prebranchial and postbranchial oxygen tensions following the exercise regime. The reduction in postbranchial oxygen tensions substantially reduced the difference between prebranchial and postbranchial oxygen tensions and thus the amount of oxygen available from physical solution, but the relatively high oxygen affinity of *Cancer magister* haemocyanin nontheless allows virtually 100% oxygen saturation of the respiratory pigment in transit through the gills. The reduction in prebranchial oxygen tension has two important consequences. Firstly, it allows a small increase in the oxygen tension gradient across the gill and hence facilitates oxygen uptake and secondly, it allows an increased release of oxygen from haemocyanin to the tissues. At pre-exercise pH (7·90), the additional release of oxygen would have been relatively small, perhaps decreasing prebranchial haemocyanin saturation below 50% (Fig. 3). However, lactic acid is released into the tissues during exercise and contributes to a marked fall in pH (table 1, McDonald *et al.* 1978). Due to the relatively large Bohr shift ($\Delta \log P_{50}/\Delta pH = 0{\cdot}785$; McDonald, 1977) over 90% of haemocyanin-bound oxygen could be released after exercise with relatively little change in P_{v,O_2} and hence no serious disruption of the haemolymph to tissue oxygen gradient. The resulting depletion of venous oxygen reserve may be of great importance in the first minutes of exercise in that it could produce rapid release of haemocyanin-stored oxygen for increased aerobic metabolism. Based on the oxygen content data of Table 1, and a haemolymph volume of 30% body mass (Alspach, 1972) this oxygen release could account for more than 50 μM O_2 or sufficient oxygen to meet the additional oxygen demand for more than 1 min. Such release might be of particular importance in short periods of strenuous activity. Modification of haemolymph oxygen binding then increases both the amount of oxygen taken up by the gills and also the efficiency of oxygen uptake by the tissues ($Extr_b$%, Table 1). Stevens & Randall (1967) were unable to demonstrate significant changes in either arterial or venous oxygen tension in moderately swimming rainbow trout and concluded that enhancement of tissue oxygen delivery occurred more by increased cardiac output than by increase in the $a\text{-}v_{O_2}$ oxygen content. More recently, however, Kiceniuk & Jones (1977) demonstrated reduction of pH and progressive increase in $a\text{-}v_{O_2}$ oxygen content difference with increasing swimming speed in this species also. Similar results were presented for the dogfish *Scyliorhinus stellaris* by Piiper *et al.* (1977), suggesting that essentially similar mechanisms are used to facilitate oxygen consumption in both crab and fish species.

In the crab several possible limitations of the gas exchange process could account for the observed shortfall in oxygen uptake during exhausting activity. Peak frequency of scaphognathite pumping usually occurred early in the exercise period, indicating that a maximum beat frequency had been reached. As the scaphognathite operates at fixed volume (McDonald, 1977) this could have limited the increase in branchial water flow. Even if this were not so, gas exchange at high ventilation rates may become less efficient. Randall (1970*b*) suggests that, in fish, anatomical, distribution and diffusion dead space all increase with increase in ventilation volume. Similar effects may have limited oxygen uptake from the increased branchial water flow observed after exercise in *Cancer magister*. Limitations could also occur in the perfusion

system. The calculated increase in stroke volume (Table 1) may approach maximum levels, and in face of the animals' apparent inability to increase cardiac output rapidly during exercise, may have limited gill perfusion. Finally, limitation might occur in the gills themselves. Gill area in this species ($4{\cdot}4 \pm 0{\cdot}2$ S.D. $cm^2.g^{-1}$ body mass: B. R. McMahon, in preparation) is within the range quoted for a variety of fish species by Hughes & Morgan (1973) but aerobic scope is less. Crustacean gills, however, contain a layer of chitin which, because of its more than 10-fold greater resistance to oxygen diffusion (Krogh, 1919) may limit the level of oxygen uptake obtained during exercise. A 10× greater figure for O_2 permeation through chitin published in Krogh (1941) is based on no new evidence and is assumed here to be a printer's error.

In *Cancer magister* the short fall in oxygen consumption during exercise is apparently accompanied by increased anaerobic metabolism. Pronounced lactic acid build up occurs in haemolymph of this species following strenuous exercise and contributes to a pronounced acidosis (Table 1). Phillips *et al.* (1977) report lactic acid build up following exercise in seven other species of Crustacea, and it appears that this may be a major route for anaerobic metabolism in these decapod Crustacea, as in many teleost fishes (reviewed by Bennett, 1978) and a single elasmobranch species (Piiper *et al.* 1972). Other anaerobic pathways however may play an important role in lower vertebrates (Wood *et al.* 1977; Hughes & Johnston, 1978), and may also be important in *Cancer* and other Crustacea.

The effects of severe physical activity are more persistent in *Cancer magister*, in other crustaceans (present study and Phillips *et al.* 1977) and in lower vertebrates, than in mammals (Bennett, 1978). In both of the former groups lactate levels continue to increase for 1–3 h following exercise and they decline only slowly, not reaching pre-exercise levels for 8–24 h. Oxygen uptake also remains elevated above pre-exercise levels for 3–10 h, but the time courses of lactate removal and oxygen debt repayment are apparently not tightly coupled since oxygen consumption returns to resting levels before lactate is completely eliminated (Bennett, 1978; McDonald, 1977). Lactate appears not to be excreted in crustaceans (C. Bridges & A. Brand, personal communication) but details of its metabolism are poorly known. The hepatopancreas does not seem to be involved in at least gluconeogenesis from lactate in decapod Crustacea (Phillips *et al.* 1977). Dreidzic & Kiceniuk (1976) suggest that the gills may play a major role in lactate metabolism in rainbow trout, and the high levels of gluconeogenesis in the isolated gills of the crab *Carcinus maenas* (Thabrew, Poat & Munday, 1971) indicate that this tissue may also be important in decapod crustaceans. As in lower vertebrates, however (Bennett, 1978), neither the site nor the manner of metabolism of lactate produced in exercise can be accurately ascertained.

The authors express their thanks to the director and staff of the Friday Harbour Laboratory, University of Washington, where some of this work was carried out. The work was supported by a National Research Council of Canada, grant no. A5762, to B. McMahon.

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