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The Physiological Properties and Function of Ventilatory Pauses in the Crab *Cancer pagurus*

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Summary. 1. The function of ventilatory pauses in the crab, *Cancer pagurus*, was investigated by analyzing pre- and postbranchial hemolymph, sampled via chronic indwelling catheters before, during and after pauses.

2. Evidence is presented that aerobic metabolism declines when ventilation ceases and that hemolymph O_2 stores are nearly depleted during a typical pause.

3. At 10 °C and 34‰ salinity, hemocyanin has a low O_2 affinity ($P_{50}=9$ Torr at pH 7.96) and a large, normal Bohr shift ($\Delta\log P_{50}/\Delta\mathrm{pH}=-0.95$). The cooperativity between O_2 binding sites is high ($n_{50}=3.5$).

4. It is suggested that pausing behavior serves to optimize the expenditure of energy during periods of non-activity by calling on O_2 stores built up during ventilating periods. *Cancer pagurus* accomplishes this by nearly completely saturating its low O_2 carrying capacity hemolymph with O_2. Having a large hemolymph volume and high P_{O_2} in the venous hemolymph pool are obvious advantages.

Introduction

The occurrence of periodic simultaneous apnoea and bradycardia among the decapod crustaceans has been observed numerous times (McMahon and Wilkens 1972; Ansell 1973; McDonald et al. 1977; McMahon and Wilkens 1977; Bridges 1979). The neural control of these respiratory pauses is fairly well understood (see Wilkens 1976). However, the functional significance of periodic cessation or reduction of respiratory movements is not clear. Recently, McMahon and Wilkens (1977) analyzed respiratory and circulatory variables during respiratory pauses in *Cancer productus*. While their findings indicate that physically dissolved and hemocyanin-bound oxygen in the hemolymph are utilized to a small degree during periods when branchial ventilation ceases and bradycardia occurs, these oxygen stores are relatively untapped.

Cancer pagurus (L.) occurs sublittorally around the coasts of Europe and exhibits rhythmic respiratory behavior (Ansell 1973). The present study was undertaken to evaluate the function of ventilatory pauses in this species using chronic indwelling prebranchial and postbranchial catheters to sample hemolymph during pausing and ventilating periods. We present evidence suggesting that aerobic metabolism declines when ventilation ceases and that hemolymph oxygen stores are nearly depleted during a typical pause. These findings indicate that pausing behavior may lead to a saving of metabolic energy in resting crabs.

Materials and Methods

Large *Cancer pagurus* (400–750 g) were collected at the Kristineberg Marine Biological Station in Kristineberg, Sweden and transported to the Zoophysiology Laboratory in Aarhus, Denmark where they were held in a large (2,000 l) recirculating sea water system at 10±1 °C and approximately 33 ‰ salinity in a 12:12 L:D cycle. Crabs were fed fresh mussels or fish at least twice each week. Both male and female crabs were used.

Branchial Ventilation and Oxygen Uptake. Branchial ventilation ($\dot{V}$w) was measured by continuously monitoring the rate of water flowing through a plastic mask placed over the crab. The mask was constructed from a flexible polymer called Drufosoft (Dreve Dentamid, Unna, Germany) and fitted to the contours of the crab with a rubber impression material ('Coe Flex,' Coe Laboratories, Inc., Chicago, USA). The mask was then made water tight using stopcock grease and held in place with rubber bands. The water flow through the mask was measured with an electromagnetic flow probe (Micron Instruments) and meter (Micron Instruments RC1000). The output of the electromagnetic flow probe was electronically integrated (Brush Gould) to provide a measure of mean

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flow. The P_{O_2} of inhalant branchial water (P_{IO_2}) was measured by siphoning water through the thermostatted chamber of a P_{O_2} electrode (Radiometer E5046 P_{O_2} electrode and PHM71 meter). The P_{O_2} of the exhalant branchial water (P_{EO_2}) was measured in a similar manner by siphoning water from the exhalant ventilation stream after it passed the electromagnetic flow probe. The time delay for changes in water P_{O_2} and detection by the P_{O_2} electrode was less than one minute. The above measurements (P_{IO_2}, P_{EO_2}, $\dot{V}w$ and integrated flow) were monitored continuously on a four channel recorder (Hewlett Packard 7404A).

Oxygen uptake ($\dot{V}_{O_2}$) was calculated by multiplying the inhalant-exhalant O_2 concentration difference by the flow rate of water through the branchial chambers at 1–3 min intervals in ventilation periods at least 7 min long. $\dot{V}_{O_2}$ was calculated from periods of normal ventilation.

Branchial Chamber Pressures and Heart Rate. To measure branchial chamber pressures and scaphognathite activity, polythene catheters were inserted into holes drilled posteriorly in each epibranchial chamber (Hughes et al. 1969). Each catheter was connected to a Statham P23BB pressure transducer and the output amplified (Brush Gould) and recorded.

Heart rate was recorded from two silver wire electrodes implanted in the lateral regions of the pericardium using an impedance technique (Helm and Trueman 1967). The electrode wires were sealed in place using cyanoacrylate adhesive and dental wax (Metrodent).

Hemolymph Sampling and Analysis. Prebranchial hemolymph samples were obtained from a catheter placed in the infrabranchial sinus at the base of the third or fourth walking leg. The catheter pierced the tough arthrodial membrane at the base of the leg, was inserted deep (3–5 mm) into the infrabranchial sinus and held in place by a rubber band glued to the carapace. Postbranchial hemolymph samples were taken via an indwelling catheter placed in the pericardial cavity. The catheter was placed through a hole drilled in the carapace and held in place with cyanoacrylate adhesive and dental wax.

The dead space of each catheter had a volume of about 100 µl and was filled with hemolymph. Before sampling hemolymph, the hemolymph occupying the dead space was removed and discarded.

Hemolymph samples were taken at various times throughout the ventilatory pause and ventilating periods. During hemolymph sampling, branchial chamber pressure and heart rate were monitored simultaneously to determine the duration of the pause. A 400 µl prebranchial hemolymph sample was taken into a 1 ml glass syringe after which a 400 µl postbranchial sample was taken. In no case did the total volume of hemolymph removed from the crab during an experiment exceed 10% of the total hemolymph volume which was assumed to be 30% of the wet weight of the animal (Gleeson and Zubkoff 1977). Syringes containing the hemolymph samples were immediately packed in crushed ice and the hemolymph analyzed for P_{O_2}, total oxygen content, pH and P_{CO_2} within an hour of sampling. Analysis of variables in hemolymph samples treated similarly, but in different species, confirmed that there was no significant difference between values obtained from hemolymph immediately after sampling from a crab and the same hemolymph one hour later (Table 1).

Hemolymph P_{O_2} was determined using a Radiometer P_{O_2} meter (PHM71) and electrode (E5046) thermostatted to 10 °C. Total oxygen content of hemolymph was measured with a technique described by Tucker (1967) and modified by Bridges et al. (1979). Hemolymph pH was determined using a Radiometer Acid-Base Analyzer (BMS2 Mk2) thermostatted to 10 °C and Radiometer pH meter (PHM64). Hemolymph P_{CO_2} was estimated using the Astrup equilibration method (Astrup 1956).

Hemolymph oxygen carrying capacity was determined by equilibrating a hemolymph sample at 10 °C with air and a P_{CO_2} approximating in vivo conditions. The appropriate gas pressures were achieved using Wösthoff gas mixing pumps (Type 1 M201/a–F and Type I M10-F). The total oxygen content of this hemolymph sample was measured as described above. Hemocyanin-bound oxygen was calculated by subtracting the quantity of oxygen physically dissolved in the hemolymph from the total oxygen content of the sample.

To determine if the hemocyanin of *C. pagurus* has special O_2 equilibrium properties which are exploited during pausing behavior, oxygen equilibrium curves were determined on undiluted hemolymph at 10 °C and different P_{CO_2} levels using the diffusion chamber method of Sick and Gersonde (1969) with the modifications used by Lykkeboe et al. (1975) and Bridges et al. (1979).

Table 1. Storing hemolymph samples in syringes packed in ice has little affect on hemolymph pH, total CO_2 content and total O_2 content after one hour in four *Cancer anthonyi* and an individual *Cancer antennarius* (all females). Variables were measured immediately after withdrawing the postbranchial sample from each crab and one hour later. Separate hemolymph samples were taken for each variable measured. Crabs were held in well-aerated sea water (34 ‰ salinity) at 20±1 °C. Data are expressed as the mean ± standard error. pH means and standard errors reflect the conversion of pH to hydrogen ion concentrations. In all cases no statistically significant differences ($P>0.05$) were noted between 0 and 1 h according to a paired t test. Data were collected by H.W. Kohl, University of San Diego

	pH	C_{CO_2} (mM)	C_{O_2} (vol. %)
0 h	7.91 (7.84–7.99)	13.39 (±1.63)	0.84 (±0.15)
1 h	7.86 (7.77–7.97)	14.16 (±1.67)	0.88 (±0.18)

Results

A rhythmic pattern of breathing, showing a periodic and spontaneous cessation of branchial ventilation, was observed in all of ten specimens of *Cancer pagurus* investigated. Heart beat frequency decreased at the onset of apnoea, while scaphognathites in both branchial chambers ceased beating (Fig. 1). On several occasions only one scaphognathite ceased beating.

Average pause duration recorded over a one-two hour period showed variation between two crabs. Mean pause duration and percent time pausing decreased with ambient P_{O_2} while the periods of active ventilation increased (Table 2). O_2 uptake rates from five animals were averaged at different times after the resumption of ventilation activity in the postpause period. O_2 uptake was elevated after each pause and gradually returned to prepause levels (Fig. 2).

The period following a pause was analyzed by measuring the rate of O_2 uptake in several crabs after numerous pauses. Some interesting generalizations can be made when these data (Fig. 2) are treated in a manner similar to O_2 uptake data obtained from diving animals before and after diving (see Dejours 1975). If gas exchange between the ambient medium and the crab ceases during the pause, the O_2 consumed during this period must come from the O_2

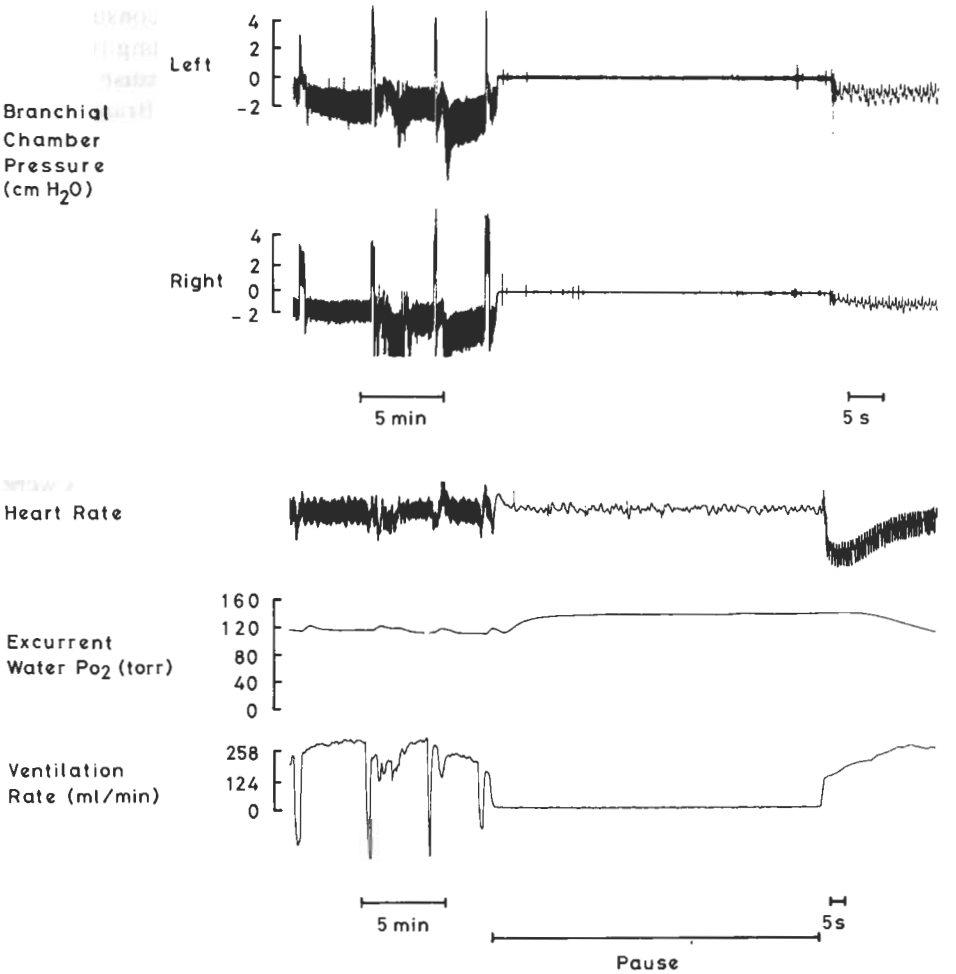

Fig. 1. Left and right branchial chamber pressure, heart rate, excurrent water P_{O_2} and ventilation in *Cancer pagurus* before, during and after a 12 min ventilatory pause at 10 °C and 33 ‰ salinity. Two different recorders were used to make these measurements. Note the different chart speeds at the end of the pause

Table 2. Pause and ventilation periods in two *Cancer pagurus* during progressive hypoxia. Data at each P_{IO_2} were obtained from a period of time 1.3–2.3 h long over 3–5 pause-interpause periods

Crab no.	P_{IO_2} (Torr)	Mean pause period (min)	Mean ventilating period (min)	Time pausing (%)
4	140–145	13.98	15.88	46.8
	66	12.3	9.37	56.8
	42	8.6	17.9	32.5
	24	4.3	27.8	13.4
5	140–145	7.84	9.86	44.3
	66	2.39	12.66	15.9
	43	5.05	22.42	18.4

stored within the crab. The assumption is made that the O_2 dissolved in the unmixed interlamellar spaces of the gills and in the branchial chambers is a negligible component of the total O_2 stores (see below). Thus, O_2 borrowed from the internal stores during a pause is a deficit which must be repaid in the post-pause period with rates of O_2 uptake from the ambient medium elevated above the prepause level. This situation is analogous to the air breathing diver which must utilize its oxygen reserves during a dive and afterwards replenish the reserves by taking up O_2 from the air at rates above the predive levels. In *C. pagurus* the O_2 taken up during the postpause recovery period to repay the O_2 debt incurred during the pause is quantified by the stippled area under the postpause curve in Fig. 2. The remaining area under this curve is attributed to normal O_2 uptake. The ratio of the O_2 utilized to repay the O_2 debt after the pause to the deficit incurred during the pause should be at least one if the O_2 consumption rate during the pause is equal to prepause levels. In this study, the ratio is 0.83. This result indicates that the rate of O_2 consumption during a pause is lower than prepause levels by at least 17%. However, O_2 consumption during a pause, as estimated by postpause O_2 debt repayment, is high since the excess O_2 used

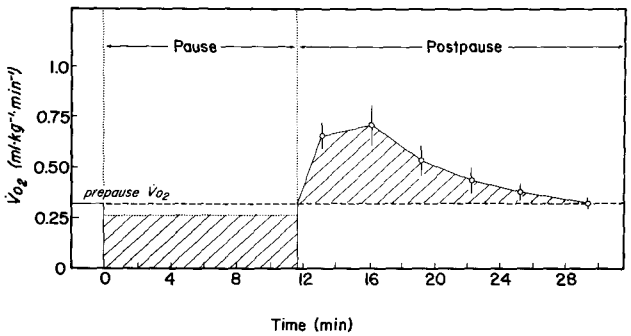

Fig. 2. Prepause and postpause O_2 uptake in *Cancer pagurus*. Mean $\dot{V}_{O_2}$ (±S.E.) were derived from three crabs over 15 pauses. Mean prepause $\dot{V}_{O_2}=0.33$ ml·kg^{-1}·min^{-1} ±0.014 S.E.; mean pause length = 11.7 min ± 1.4 S.E. The stippled area in the pause region represents the quantity of oxygen used from oxygen stores as predicted by the oxygen taken up during the recovery period in excess of the amount normally consumed

to repay the deficit includes any O_2 consumed to oxidize accumulated end products resulting from anaerobic energy production during the pause (lactate is apparently not excreted; Bridges and Brand 1980). Additional O_2 must also be taken up to fuel the extra energy consumed by the scaphognathites to hyperventilate the branchial chambers in the period immediately following a pause. Thus, the O_2 consumed during a pause is probably smaller than the 83% of prepause levels.

An independent estimate of O_2 consumption during a pause was obtained for two crabs by measuring the decline in hemolymph oxygen content at different times during a pause (Fig. 3, hemolymph O_2 content not shown, and Fig. 4). O_2 consumption estimates were made assuming that hemolymph O_2 stores were the only available source of oxygen for respiring tis-

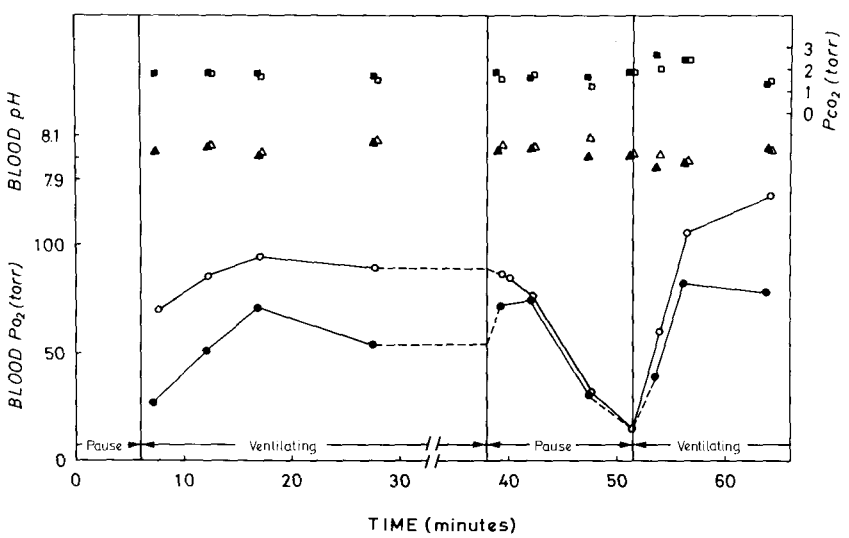

Fig. 3. Prebranchial (*closed symbols*) and postbranchial (*open symbols*) hemolymph pH, P_{CO_2} and P_{O_2} in *Cancer pagurus* (crab no. 3) during pausing and normal ventilating behavior. The dashed lines indicate the interpolation of the data

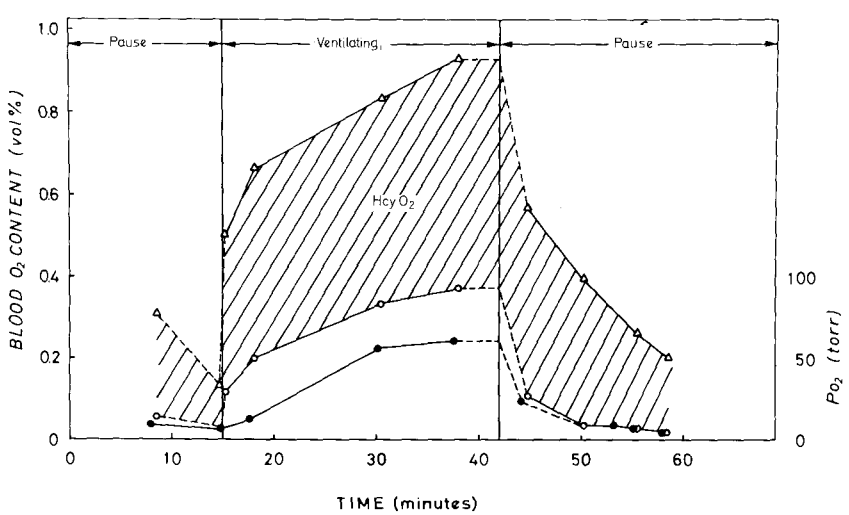

Fig. 4. Prebranchial (●) and postbranchial (○) hemolymph P_{O_2} and postbranchial hemolymph O_2 content (△) during pausing and normal ventilating behavior in crab no. 7. The stippled area represents the quantity of oxygen bound to hemocyanin while the area below the hemolymph P_{O_2} data points represents the quantity of oxygen physically dissolved in the hemolymph

sues. The O_2 dissolved in the unmixed gill interlamellar water in the branchial chambers was considered to be negligible. The possibility that gill interlamellar branchial water exchanged with water in the branchial chambers surrounding the gills providing a basis for oxygen exchange was ruled out since we never observed sudden declines in $P_{E_{O_2}}$ immediately upon resumption of ventilation after a pause. The decline in hemolymph O_2 content with time, multiplied by the hemolymph volume of the crab (30% body weight based on hemolymph volume in a portunid crab; Gleeson and Zubkoff 1977), yielded values of O_2 consumption averaging 0.11 ml kg^{-1} ($\pm$0.025 S.E.; N=3) in one crab and 0.078 ml kg^{-1} min^{-1} ($\pm$0.008 S.E.; N=3) in another. These values are 33% and 24%, respectively, of O_2 uptake measured in other actively ventilating crabs just prior to a pause (0.33 ml kg^{-1} min^{-1} $\pm$0.014 S.E.; N=16). These calculations are based on the premise that hemolymph continues to circulate during a pause. Indirect evidence for hemolymph flow was obtained by heart impedance records similar to that shown in Fig. 1, indicating the movement of the heart muscles.

The two methods yield quite different values for O_2 consumption during a pause. Importantly, both methods indicate a reduction in aerobic metabolism. The magnitude of the overall reduction probably lies somewhere between 24 and 83% of prepause O_2 consumption.

Hemolymph oxygen pressures were measured during pauses in five different crabs. In three crabs both pre- and postbranchial hemolymph P_{O_2} was measured (see Figs. 3 and 4 for 2 examples) while in the remaining two crabs only prebranchial or postbranchial hemolymph P_{O_2} was measured. In each case P_{O_2} dropped to low levels during a pause. P_{O_2} differences between prebranchial and postbranchial hemolymph were negligible several minutes into a pause (Figs. 3 and 4). Hemolymph pH and P_{CO_2} showed no statistically significant change before, during and after a pause (N=4 including Fig. 3), although there was a slight acidosis and a slight increase in P_{CO_2} associated with the initial part of the postpause recovery period. Hemocyanin was fully oxygenated in postbranchial hemolymph during a ventilating period, while after 16 min of a 28 min pause total hemolymph oxygen content fell from 0.92 vol.% to about 0.2 vol.% (Fig. 4).

Oxygen equilibrium curves were determined spectrophotometrically at three different P_{CO_2}'s and therefore three different pH levels (Fig. 5A). The in vivo oxygen equilibrium curve (Fig. 5B), constructed by measuring the P_{O_2} and oxygen content of hemolymph freshly sampled during various stages of a pause, is

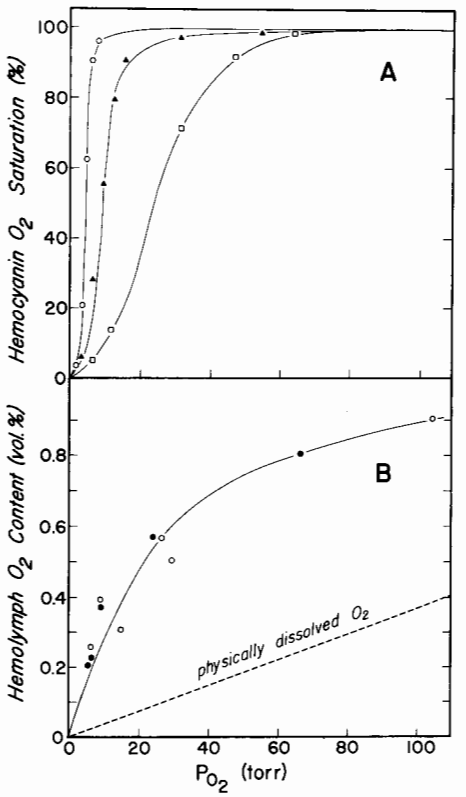

Fig. 5A, B. Oxygen equilibrium curves for *Cancer pagurus* hemocyanin at 10 °C and 33 ‰ salinity. **A** Oxygen equilibrium curves of undiluted hemolymph at three different pH's determined using the diffusion chamber (□=pH 7.635, P_{CO_2} 12.0; ▲=pH 7.968, P_{CO_2} 2.25; ○=pH 8.230, P_{CO_2} 0.75). **B** An oxygen equilibrium curve determined by measuring total O_2 content and P_{O_2} in hemolymph sampled before, during and after apnoea in crab no. 7 (see Fig. 4). Mean pH=7.96. Prebranchial (●); postbranchial (○)

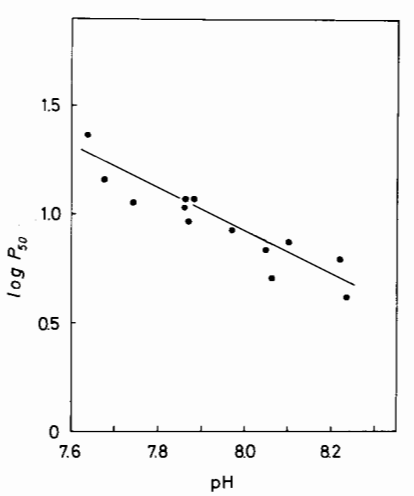

Fig. 6. The oxygen affinity (P_{50}) of *Cancer pagurus* hemocyanin as a function of pH (10 °C and 33 ‰ salinity). The magnitude of the Bohr effect, indicated by the slope of the fitted regression line, is −0.95

in close agreement with the in vitro curve at the same pH (7.96). The P_{50} in both cases is approximately 9 Torr. The hemocyanin has a large normal Bohr shift ($\Delta \log P_{50}/\Delta pH = -0.95$) between pH 7.6 and 8.2 (Fig. 6). The cooperativity between O_2 binding sites is high, shown by the n_{50} value of 3.5 ($\pm$ 0.12 S.E.; $N=13$).

Discussion

Evidence is presented that the crab, *Cancer pagurus*, fully utilizes both chemically bound and physically dissolved oxygen stores in a large hemolymph volume when ventilation ceases during bilateral pausing behavior. A decline in aerobic metabolism occurs during a pause, which inevitably lengthens the amount of time a crab may maintain this behavior. The cause for this decrease in aerobic metabolism must be related, in part, to the decrease in energy expended by the crab to pump water across the gills and hemolymph through the open circulatory system, since scaphognathite activity ceases and heart rate declines.

The prebranchial P_{O_2}'s we observed during normal ventilation (Figs. 3 and 4) are much higher than values previously reported for crabs at a similar temperature (Johansen et al. 1970; McMahon and Wilkens 1977; McMahon et al. 1979; Houlihan et al. 1980). These differences may reflect a genuinely higher prebranchial P_{O_2} characteristic of pausing behavior in *Cancer pagurus*. However, another explanation of a high prebranchial P_{O_2} may be that our sampling technique removed hemolymph not representing a truly mixed venous sample. The possibility remains that hemolymph was sampled from an inactive limb (the third walking leg) which would theoretically yield a higher P_{O_2}. We cannot exclude this possibility. However, in three individual *C. pagurus* in which we had implanted catheters we obtained low values of Pv_{O_2} (16, 16.5, 24.5) as well as high values (43.5, 44, 53.5, 66). In these crabs there was no obvious correlation between limb activity and Pv_{O_2}. While we cannot rule out other explanations for high prebranchial P_{O_2}, we feel they are representative of prebranchial P_{O_2} found in spontaneously pausing individuals of this species.

The convergence of the values for prebranchial and postbranchial hemolymph P_{O_2} during a pause is indicative of the lack of gill utility as an O_2 exchanger during this time. However, it is possible that hemolymph from the prebranchial and postbranchial sampling sites was drawn, in part, from the same hemolymph pool. This is likely only if hemolymph can somehow bypass the gills. Although shunting of hemolymph around the gills has never been demonstrated it cannot be ruled out. In the absence of any type of shunt, large gill hemolymph volumes (Burnett and Woodson, unpublished data from *Cancer anthonyi*) separating the prebranchial and postbranchial hemolymph compartments would preclude sampling from the same hemolymph pools.

We did not measure hemolymph lactate concentrations in any of our experiments and so we are unable to evaluate the contribution of anaerobic components to the overall metabolism. However, the lack of any significant hemolymph pH change both during and after a pause ($N=4$ including Fig. 3) is indirect evidence that if an anaerobic component is present, it is small.

The lack of a change in hemolymph pH is, in itself, an interesting phenomenon. During a ventilatory pause where gas exchange with the medium ceases, a hemolymph respiratory acidosis is expected due to an accumulation of CO_2. That such a change does not occur is not surprising if aerobic metabolism, and thus CO_2 production, is sufficiently depressed during a pause as illustrated in the following example.

During a 12 min ventilatory pause, CO_2 production, calculated using prepause O_2 uptake levels and assuming an RQ of 0.9, would induce an increase in total hemolymph CO_2 concentration of 1.8 mmol l^{-1} for a 500 g crab. Using the Henderson-Hasselbalch equation, constants for crab hemolymph (Truchot 1976) and the in vitro buffer line for *C. pagurus* hemolymph (determined by Astrup equilibration), an increase in hemolymph P_{CO_2} from 1.9 to 3.9 Torr and a decrease in pH from 8.0 to 7.75 are predicted. However, if we assume that the average O_2 consumption rate for a crab during a pause is about one-third the prepause level then we calculate an increase in total hemolymph CO_2 of 0.67 mmol l^{-1} corresponding to an increase in hemolymph P_{CO_2} to 2.5 Torr and a decrease in pH to 7.89, much smaller changes.

Although the quantity of O_2 stored in postbranchial hemolymph is not large (Fig. 4), it is somewhat enhanced by the crab's large hemolymph volume. Furthermore, the presence of hemocyanin in *C. pagurus* imparts an important O_2 storage function to its hemolymph. It is clear that without hemocyanin, ventilatory pauses could last no longer than one to three minutes even when O_2 consumption is reduced by 1/3.

These results contrast those of McMahon and Wilkens (1977) who demonstrated that postbranchial hemolymph P_{O_2} in *Cancer productus* remained high (about 70 Torr) after ten minutes of pausing. The differences between their data and ours probably reflect the different techniques used for sampling hemolymph. McMahon and Wilkens (1977) were unable to obtain serial samples without disturbing the animals. They therefore sampled hemolymph from three different animals over many pauses. The present study

utilized chronically implanted catheters to sample hemolymph before, during and after a pause. The maintenance of patent catheters was extremely difficult due to the formation of clots which blocked the catheters. The application of this technique in this instance was successful only after numerous trials and was aided by operating at a low ambient temperature (10 °C).

The O_2 equilibrium properties of *C. pagurus* hemocyanin are similar to those found in the same species by Truchot (1971) who reported a Bohr effect of -1.0 for the same pH range and an n_{50} value of 3.67, but at 15 °C. These properties are similar to those reported for a variety of other crustaceans (see Mangum 1980). Thus, *Cancer pagurus* emerges as a 'typical crustacean' with low hemocyanin concentrations in its hemolymph serving both O_2 transport and O_2 storage functions. It appears that all crustaceans possessing an O_2 carrier should be capable of exhibiting pausing behavior and in fact, ventilatory pausing has been observed numerous times among crustaceans (McMahon and Wilkens 1972; Ansell 1973; McMahon and Wilkens 1977; McDonald et al. 1977).

The significance of ventilatory pausing behavior in crustaceans remains unclear. McMahon and Wilkens (1972, 1977), Florey and Kriebel (1974) and Wilkens et al. (1974) have suggested that pausing behavior is related to a defensive role in concealing the crab from predators or related to a startle response. The evidence presented in this study suggests that another function of pausing behavior is energy conservation. If energy utilization by aerobic pathways in a resting crab declines by at least 50% during pausing behavior, then about 20–25% of the total energy normally consumed in a resting crab can be conserved if 40–50% of the time is spent pausing. Such a strategy may be important energetically in lowering the costs for an animal living in an environment where food resources are not abundant.

During hypoxia the pauses are shorter and the time spent pausing is reduced. This result is very similar to the one obtained by McMahon and Wilkens (1977) for *C. productus* and is most likely related to the smaller stores of physically dissolved O_2 in the hemolymph. The O_2 affinity of hemocyanin in crustaceans permits the storage of significant quantities of oxygen in the hemolymph even when the hemolymph P_{O_2} is very low, especially when a hypoxia-induced hemolymph alkalosis raises the pigment O_2 affinity (McMahon et al. 1978; Burnett 1979; Burnett and Johansen 1981).

Although hemolymph O_2 stores are reduced, it is still adaptive for a crab to pause during hypoxia. A typical response of crustaceans to hypoxia is to increase the flow of water through the branchial chambers (McMahon and Wilkens 1975; Batterton and Cameron 1978; Butler et al. 1978; Burnett 1979). Although the energetic cost of the compensatory hyperventilation must be greater than that during normal ventilatory flow, it cannot be evaluated at this time. However, a cessation of ventilatory movements during a pause in hypoxic water would still lead to substantial energy savings because the branchial chamber pumps are even more expensive to run compared with the pumps in normoxic crabs.

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