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AN ANALYSIS OF ACID-BASE DISTURBANCES IN THE HAEMOLYMPH FOLLOWING STRENUOUS ACTIVITY IN THE DUNGENESS CRAB, *CANCER MAGISTER*

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SUMMARY

Enforced activity causes a marked depression of haemolymph pH in *Cancer magister*. Both lactate concentration and P_{CO_2} of the haemolymph are elevated immediately following exercise but resting P_{CO_2} is restored within 30 min whereas resting lactate levels are not restored for at least 8 h. The haemolymph acid-base disturbance is caused largely by elevated haemolymph lactate levels but a Davenport analysis based on measurements of pH and total CO_2 reveals a marked discrepancy between the amount of metabolic acid buffered by the haemolymph and the lactate anion concentration. This appears due to a more rapid release of lactate from the tissues than H^+ ions produced with lactate.

INTRODUCTION

Strenuous activity causes a marked depression in haemolymph pH in decapod crustaceans (Johansen, Lenfant & Mecklenberg, 1970; Mangum & Wieland, 1975; Phillips *et al.* 1977). Although the nature of this acidosis has not been examined in crustaceans it may, by analogy with vertebrate studies, have both respiratory and metabolic acid (lactic acid) components. Two recent studies on marine fish offer differing views of the relative contributions of these components to the depression of blood pH following exercise. In the elasmobranch *Scyliorhinus stellaris* (Piiper, Meyer & Drees, 1972) most of the acid-base disturbance was attributable to an increase in blood lactic acid, a situation similar to that in mammals. Unlike mammals, a large deficit developed in the recovery period between calculated blood levels of metabolic acid and measured blood lactate concentrations. Piiper *et al.* (1972) thus suggested that a significant portion of the H^+ ions produced with lactate were not immediately excreted into the blood but were initially retained and buffered intracellularly.

In contrast in the starry flounder *Platichthys stellatus* the acidosis following exhaustive exercise was due largely to elevated P_{CO_2} levels (Wood, McMahon & McDonald, 1977). In this animal blood lactic acid contributed significantly to the

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acidosis only late in the recovery period. Furthermore it was apparent that all H^+ ions produced with lactate contributed to the observed acidosis. Wood *et al.*, in fact, postulated that an additional metabolic acid (possibly ammonium) may also have contributed to the pH drop during the recovery period.

The purpose of the present study was to investigate acid-base disturbances following exercise in a marine crustacean by monitoring haemolymph pH, total CO_2 and lactate and to quantify, using analytical procedures similar to those in the above studies, the relative contributions of lactic acid and P_{CO_2} to the observed acidosis. *C. magister* was chosen for study because of its large size and because of the relative ease with which haemolymph samples can be repetitively withdrawn.

MATERIALS AND METHODS

Adult Dungeness crabs (*C. magister* Dana; 490 to 1030 g) in intermolt stage C_4 (Drach & Tchernigovtzeff, 1967) were obtained from the Vancouver Public Aquarium and held for at least 2 weeks before use in large sandy bottomed tanks in the marine aquarium facility at the University of Calgary. The acclimation conditions were those employed in subsequent experiments; recirculating, filtered sea water, salinity = 27 ± 1‰, temperature = 8 ± 1 °C.

I. In vivo *experiments*

For all *in vivo* experiments the crabs (N = 7) were restrained in air while post-branchial (i.e. arterial) sites were prepared for haemolymph sampling. Small holes were drilled in the dorsal carapace so as to expose the epidermis overlying the anterio-lateral corners of the pericardial sinus. These two holes were sealed with a rubber membrane (dental latex) glued to the carapace with cyanoacrylate glue. In three crabs pre-branchial (i.e. venous) sites were similarly prepared by gluing dental latex to the arthrodial membranes at the bases of either the third or fourth walking legs. The animals were then transferred to individual chambers (18 × 30 × 10 cm deep) and allowed to recover for at least 72 h before haemolymph sampling was begun.

Haemolymph was withdrawn from the prepared sites with a 1 ml glass syringe and 23 gauge needle. The dead volume of the syringe was filled with Millipore (0·22 μm) filtered seawater and the syringes were chilled on ice prior to sampling. Post-branchial haemolymph samples (0·4–0·5 ml) could be routinely withdrawn without apparent disturbance to the animal. Sampling was alternated between the two post-branchial sites to minimize the development of clots within the pericardial cavity. In preliminary experiments (McDonald, 1977) it was determined that haemolymph samples drawn from the limb sinuses accessible through the arthrodial membranes represented mixed prebranchial haemolymph. These samples were somewhat more difficult to obtain and usually involved moving the animal to insert the sampling needle. Consequently pre-branchial samples were taken immediately following post-branchial samples and smaller volumes (0·1–0·2 ml) were drawn.

One to two haemolymph samples were withdrawn before the crab was provoked into activity. Animals were exercised for 20 min, initially by periodic prodding and then subsequently more severely manipulated by hand to ensure near continuous

motion. By the end of the exercise period the crabs were normally refractory to stimulation. Post-branchial haemolymph samples were drawn immediately post-exercise (time 0) and then at 30 min, 1 h, 2 h, 4 h, 8 h, 24 h, 48 h and 72 h. Pre-branchial samples were drawn at time 0, +30 min, +1 h and +4 h.

II. In vitro *experiments*

In vitro determinations of CO_2 combining curves and haemolymph buffer capacities (Δ $[HCO_3^-]+[CO_3^=]/\Delta$ pH) were performed by a method similar to that of Truchot (1976*b*) on haemolymph drawn from each crab at the termination of the *in vivo* experiment. Four to six ml of haemolymph were withdrawn from the pericardial sinus, shaken to promote clotting, centrifuged at 5000 *g* for 10 min to remove the clot and then transferred to a round-bottomed tonometer mounted in a temperature controlled (8 °C) water bath. The tonometer was spun to distribute haemolymph on its walls and was gassed with humidified mixtures of CO_2 in air supplied by a Wösthoff gas mixing pump. Haemolymph was equilibrated (1 h) to 4–5 different levels of CO_2 in air (P_{CO_2} 0·65 to 6·5 torr). To minimize equilibration time bovine carbonic anhydrase obtained from Sigma, St Louis (final concentration 0·25 g l^{-1}) was added to the haemolymph (Truchot, 1976*b*).

III. *Analytical procedures*

All *in vivo* post-branchial samples were analysed for total CO_2, pH and lactate and in two experiments haemolymph P_{CO_2} was also determined. Pre-branchial and *in vitro* haemolymph samples were analysed for pH and total CO_2. Haemocyanin oxygen carrying capacity, $C^{max}_{Hcy}O_2$ was determined from measurements of oxygen content of haemolymph air equilibrated *in vitro*.

Haemolymph pH was measured on 40–60 μl samples injected into a Radiometer pH electrode thermostatted to the experimental temperature. The pH value was displayed on a Radiometer PHM71 acid-base meter. The pH measurement system was calibrated with Radiometer precision buffers (S1500 and S1510) having values of 6·928 and 7·475 at 8 °C. The linearity of this instrument at higher pH was checked periodically with a Sigma precision buffer (Trizma 7·4); pH 7·88 at 8 °C. Total CO_2 content of haemolymph was determined on 70–80 μl samples by the method of Cameron (1971). Each sample was bracketed with sodium bicarbonate standards to increase the accuracy of these determinations. Haemolymph lactate concentrations were determined on 0·25 ml of haemolymph. This volume was immediately deproteinated in 0·5 ml of ice-cold perchloric acid and then centrifuged at 5000 *g* for 10 min. The supernatant was analysed enzymatically (lactic dehydrogenase) for L-lactate with Sigma reagents (see Sigma bulletin no: 826-UV). Haemolymph P_{CO_2} was measured on 0·2 ml samples with a thermostatted Radiometer P_{CO_2} electrode fitted with a thin (25 μl) silicone membrane and connected to a second PHM71. At 8 °C 8–10 min was required for the electrode response. This, plus the low P_{CO_2} levels in crab haemolymph (1–4 torr) may mean that the measurements of P_{CO_2} may be in error by as much as ±20%. $C^{max}_{Hcy}O_2$ was calculated from measurements of haemolymph oxygen content on air-equilibrated samples according to the formula:

$$C^{max}_{Hcy}O_2 = C_{O_2} - \alpha \text{ diss } O_2 . P_{O_2}$$

where C_{O_2} is the haemolymph oxygen content measured on 80 μl aliquots injected into a Lex-O_2-Con oxygen content analyser, α diss O_2 is the physical solubility of oxygen in haemolymph in vols % $torr^{-1}$ (from Truchot, 1971) and P_{O_2} is the oxygen partial pressure of air-equilibrated haemolymph.

IV. *Theoretical approach*

CO_2 dissociation in the haemolymph is described by the Henderson-Hasselbalch equation:

$$pH = pk_1' + \log \frac{[HCO_3^-]}{[\alpha CO_2 . P_{CO_2}]} = pk_2' + \log \frac{[CO_3^=]}{[HCO_3^-]},$$

where pk_1' and pk_2' are the negative logarithms of the apparent first and second dissociation constants of H_2CO_3 and HCO_3^- respectively and αCO_2 is the solubility coefficient of CO_2 in haemolymph. Values for αCO_2, pk_1' and pk_2' at the experimental temperature and salinity were obtained from alignment nomograms constructed for the haemolymph of the shore crab *Carcinus maenas* (Truchot, 1976*a*).

The close correspondence found between measured values of P_{CO_2} and values calculated using the Henderson-Hasselbalch equation confirms the applicability of these constants to acid-base calculations on *C. magister* haemolymph. The mean difference between measured and calculated values was 0.24 ± 0.18 (S.D.) torr (N = 20). Paired '*t*' test analysis of measured and calculated values showed that they were not significantly different. Only calculated values for P_{CO_2} are presented in the text as the errors inherent in P_{CO_2} measurements (described above) are likely greater than errors in measurements of either total CO_2 or pH on which P_{CO_2} calculations are based.

In acid-base studies on vertebrates the dissociation of HCO_3^- to H^+ and $CO_3^=$ is usually ignored as negligible. At the high pH and ionic acitivity of crustacean haemolymph this dissociation is not negligible but at pH 7.9, $[HCO_3^-]$ exceeds $[CO_3^=]$ by a ratio of approximately 30:1. Thus, bicarbonate concentrations calculated from the Henderson-Hasselbalch equation are referred to as $[HCO_3^-]+[CO_3^=]$.

Increases in P_{CO_2} and in the concentration of acid metabolites (e.g. lactic acid) in the haemolymph will increase the quantity of H^+ ions buffered by the haemolymph bicarbonate and non-bicarbonate (mainly protein) buffer systems. By the use of a Davenport diagram (pH vs $[HCO_3^- + CO_3^=]$; Davenport, 1974) constructed specifically for the haemolymph of the crab the quantities of H^+ ions added by respiratory acids (ΔH^+c) and by metabolic acids (ΔH^+m) were separately estimated from *in vivo* measurements of haemolymph pH and bicarbonate concentrations and *in vitro* determinations of the haemolymph buffering power ($\Delta[HCO_3^-]+CO_3^=]/\Delta$ pH). This is the standard method originally designed for estimating metabolic and respiratory acid levels in human blood and is described in detail in Woodbury (1974) and Wood *et al.* (1977). This method is based on physico-chemical principles which should be common to any binary buffer system (i.e. protein plus bicarbonate buffers) and thus should be equally valid when applied to the crab system since accurate values for pk_1' and αCO_2 of crustacean haemolymph are available (Truchot, 1976*a*).

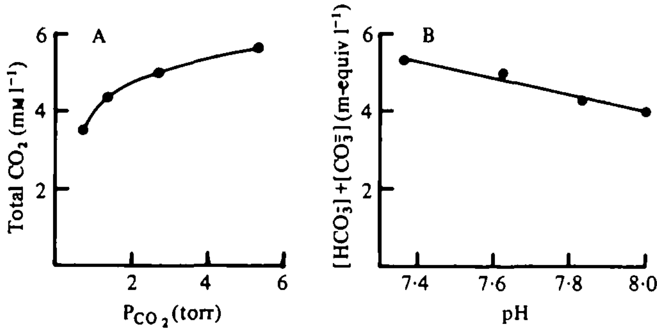

Fig. 1. (A) CO_2 combining curve and (B) haemolymph buffer curve determined *in vitro* on a haemolymph sample having a haemocyanin oxygen carrying capacity, $C^{max}_{Hcy}O_2$, of 0·52 vols %.

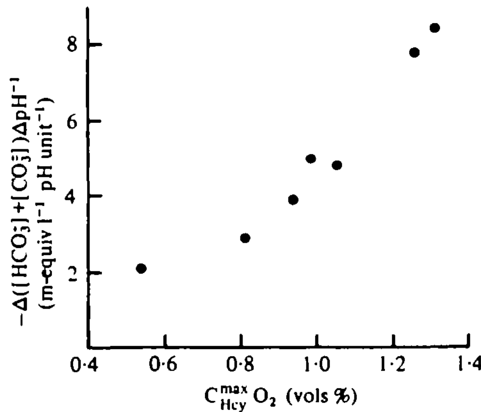

Fig. 2. Buffer values ($-\Delta[HCO_3^- + CO_3^=]\,\Delta pH^{-1}$) as a function of haemocyanin oxygen carrying capacity, $C^{max}_{Hcy}O_2$.

RESULTS

(1) In vitro *experiments*

In the crabs (N = 7) used in this study haemocyanin oxygen carrying capacity $C^{max}_{Hcy}O_2$, ranged from 0·52 to 1·31 vols %. A CO_2 combining curve determined *in vitro* on haemolymph with a $C^{max}_{Hcy}O_2$ of 0·52 vols % is illustrated in Fig. 1A. The form of this curve is typical, the only variation being a tendency for upward displacement of the curve with increased $C^{max}_{Hcy}O_2$.

The buffering power of the non-carbonate (mainly protein) buffers in the haemolymph is defined as the negative slope of the $[HCO_3^-]+[CO_3^=]$ vs. pH line. Fig. 1B shows the calculated $[HCO_3^-]+[CO_3^=]$ values for the haemolymph sample of Fig. 1A as a function of pH. Within individual crabs these values always varied linearly with pH in the range 7·3 to 8·0, thereby giving a constant buffer value. However in different crabs the buffer value was dependent upon the $C^{max}_{Hcy}O_2$ level (Fig. 2).

II. In vivo *experiments*

Following operative procedures in air crabs were initially active in their individual chambers. By 72 h they had acclimated to the experimental conditions and exhibited

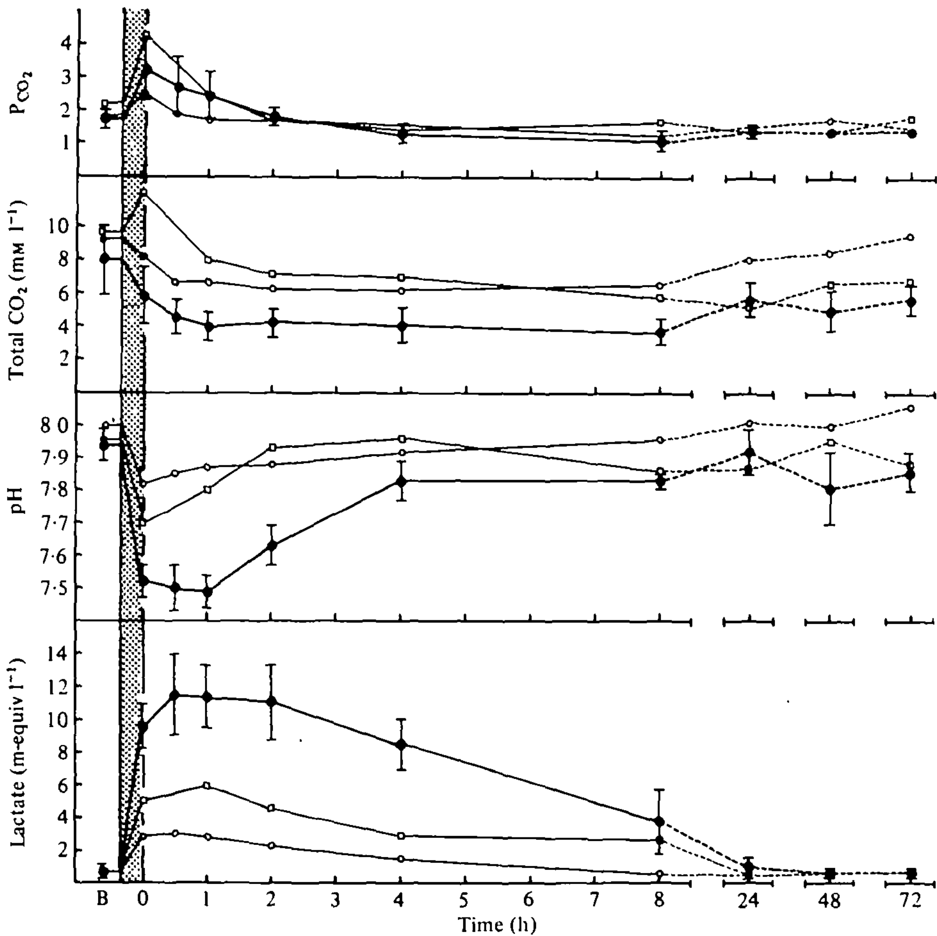

Fig. 3. P_{CO_2}, total CO_2, pH and lactate concentration in post-branchial haemolymph of *C. magister* prior to and following 20 min of enforced activity. Solid symbols are means ± one S.D. (N = 5) for male crabs (weight range 700–1030 g). Open symbols depict acid-base changes for two female crabs (□ 490 g, ○ 551 g). B = resting sample. Shaded area = exercise period. Time 0 = immediately post-exercise.

very little spontaneous activity. Post-branchial haemolymph samples drawn prior to exercise were uniform; pH averaged 7·94+0·05 (S.D., N = 7), lactate 0·69 ± 0·42 m-equiv l^{-1}, total CO_2 8·3 ± 1·8 mM l^{-1} and P_{CO_2} 1·8 ± 0·3 torr. Twenty minutes of enforced activity induced changes in all these parameters (Fig. 3). The changes were qualitatively similar in all animals but were much more marked in the males (N = 5, mean weight 845 ± 122 g) than in the females (N = 2, weights 490 and 551 g) so results for the two females are presented separately in Fig. 3.

In the male crabs Pa_{CO_2} increased from 1·7 ± 0·3 to 3·2 ± 0·9 torr during the exercise period and thereafter declined steadily reaching pre-exercise values within 2 h. Haemolymph pHa declined sharply during exercise and continued to decrease for up to 1 h post-exercise. Similarly haemolymph total CO_2 declined steadily during and following exercise reaching a minimum value at 1 h. Haemolymph lactate increased by about 11 fold during the exercise period and continued to increase for 1–2 h.

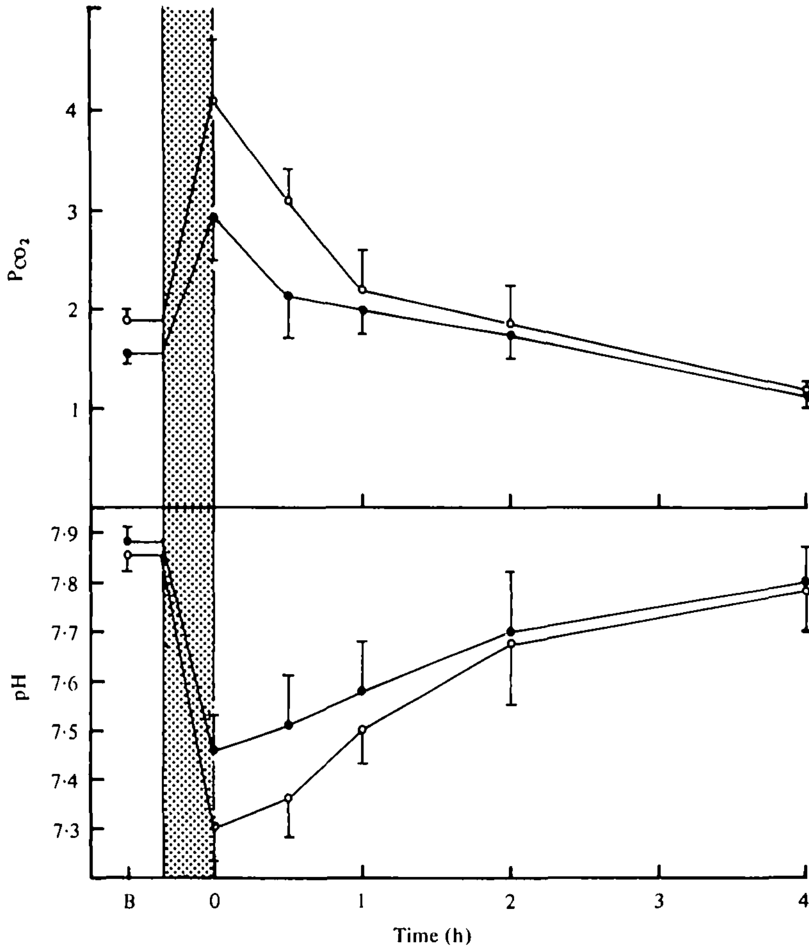

Fig. 4. P_{CO_2} and pH in pre-branchial (○) and post-branchial (●) haemolymph prior to and following 20 min of enforced activity. Values are means plus or minus one S.D. (N = 3). B = resting sample. Shaded area = exercise period. Time 0 = immediately post exercise.

Subsequently lactate concentration gradually declined until by 24 h normal resting levels were restored. The initial decreases in pHa and total CO_2 were correlated with the rise in haemolymph lactate but the recovery time courses were markedly different. Post-branchial pH had recovered to near normal levels by 4 h whereas lactate had fallen only 26% from the peak post-exercise concentration. Total CO_2 stabilized at a level significantly below resting from 1–8 h even though lactate concentrations steadily declined during this time. Total CO_2 levels subsequently increased but by 72 h post-exercise, resting levels were not completely restored.

The smaller changes in haemolymph lactate concentration in the two female crabs (Fig. 3) suggests that anaerobic metabolism played a lesser role in enforced activity in these small animals. Consequently the changes in post-branchial pH and total CO_2, both of which would be depressed by the addition of metabolic acid to the haemolymph, were also much smaller in the females.

Prior to exercise there were only slight differences in pH and P_{CO_2} between pre- and

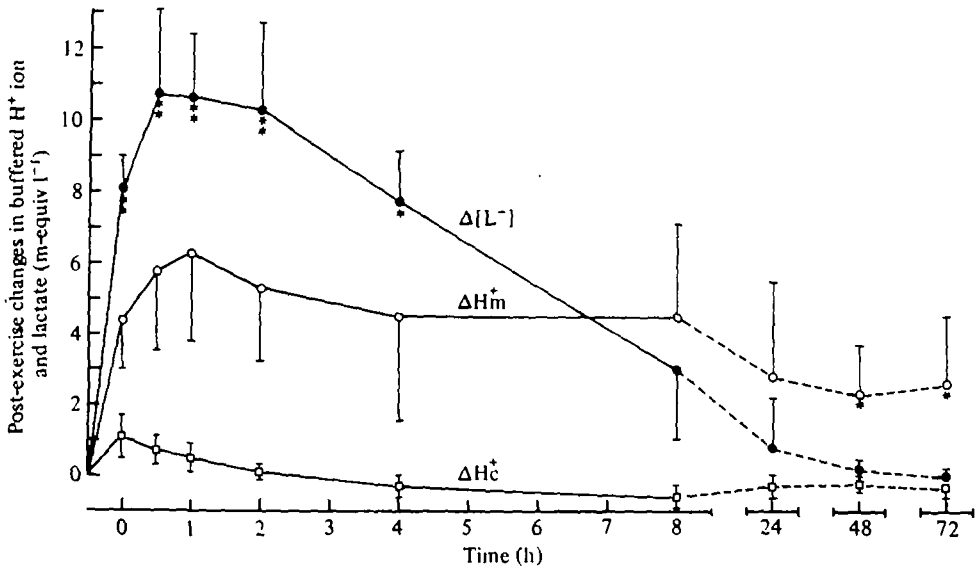

Fig. 5. Changes in lactate and buffering of H^+ ions in post-branchial haemolymph following 20 min of enforced activity. ΔH^+c is the changes in buffering of H^+ ions due to P_{CO_2}. ΔH^+m is the changes in buffering of H^+ ions due to metabolic acid (ie lactic acid) and $\Delta[L^-]$ is the changes in haemolymph lactate anion concentration. Values are means plus and/or minus one S.D. for male crabs (N = 5). Significant differences between ΔH^+m and $\Delta[L^-]$ by paired '*t*' test are indicated by asterisks, * $P < 0.05$, ** $P < 0.01$.

post-branchial haemolymph (Fig. 4). Pre-branchial pH was, in the 3 crabs examined, consistently lower by 0·02 units, on average, than post-branchial pH and P_{CO_2} was 0·3 torr higher. Post-exercise variations in pre-branchial pH and P_{CO_2} were qualitatively similar to those in post-branchial haemolymph although the absolute changes were greater. Immediately following exercise pre- and post-branchial differences reached maximal values with pre-branchial pH 0·16 units, on average, lower and pre-branchial P_{CO_2} 1·3 torr higher. Subsequently these differences diminished and the return to normal values followed similar time courses to those in post-branchial haemolymph.

The post-exercise increases in H^+ ion in post-branchial haemolymph contributed directly from a metabolic acid (ΔH^+m) and from the dissociation of H_2CO_3 (ΔH^+c) were calculated from *in vivo* measurements of pH and total CO_2 and *in vitro* determinations of the buffer value as outlined in Methods. The analysis was performed separately for each animal employing the buffer value determined specifically for its haemolymph. The results of this analysis on the five male crabs are depicted in Fig. 5. The results from the two female crabs have not been included as these animals exhibited substantially lower though qualitatively similar changes in ΔH^+m.

Throughout the post-exercise period ΔH^+c was small relative to ΔH^+m reaching a peak of $1 \cdot 1 \pm 0 \cdot 61$ m-equiv l^{-1} immediately following exercise. By 2 h post-exercise ΔH^+c had fallen to zero (resting P_{CO_2} levels restored). The bulk of the increase in H^+ ions buffered by post-branchial haemolymph was caused by an increase in metabolic acid. The peak in this contribution, occurring at 1 h post-exercise, approximately corresponded to the peak lactate anion concentration, $\Delta[L^-]$. There was, however, a considerable quantitative discrepancy. At 1 h post-exercise $\Delta[L^-]$ exceeded ΔH^+m

by 4.33 ± 0.79 m-equiv l^{-1}. Subsequently the levels of both declined in post-branchial haemolymph with $\Delta H^{+}m$ diminishing at a lower rate until by 8 h $\Delta H^{+}m$ exceeded $\Delta[L^{-}]$ by 1.51 ± 1.20 m-equiv l^{-1}.

The same analytical procedure was used to evaluate increases in carbonic and metabolic acid levels in pre-branchial haemolymph. This analysis showed that immediately following exercise the total additional amount of H^{+} ions buffered in pre-branchial haemolymph ($\Delta H^{+}c + \Delta H^{+}m$) exceeded the amount buffered in post-branchial haemolymph by 0.80 ± 0.26 m-equiv l^{-1}. Of this amount, 0.47 m-equiv l^{-1} was contributed by metabolic acid and the remainder by carbonic acid. By 1 h the total difference had decreased to 0.55 ± 0.26 m-equiv l^{-1}, of which 0.27 m-equiv l^{-1} was metabolic acid, and by 2 h the difference had decreased to 0.12 ± 0.08, all of which can be accounted for by the elevated P_{CO_2} in pre-branchial haemolymph, i.e. $\Delta H^{+}m$ in pre-branchial haemolymph was equal to that in post-branchial haemolymph.

DISCUSSION

A common characteristic of the haemolymph of decapod crustacea is a large intra-specific variability in protein concentration (Horn & Kerr, 1963; Uglow, 1969; Busselen, 1970; Truchot 1976*b*). Truchot (1976*b*) has shown in the shore crab *Carcinus maenas* that the buffering power of the non-bicarbonate buffers in the haemolymph is closely correlated with protein concentration and also with the haemocyanin oxygen carrying capacity, $C^{max}_{Hcy}\,O_2$. Truchot thus concludes that the main buffers are the haemolymph proteins, principally the respiratory pigment haemocyanin. In *C. magister* we find a similar correlation; a more than two-fold range in $C^{max}_{Hcy}\,O_2$ results in a four-fold range in haemolymph buffering power (Fig. 2).

Interestingly, this variation in buffer capacity has no apparent effect on the magnitude of the acidosis provoked by 20 min of activity. In the male crabs (buffer capacities 2.1 to 8.4 m-equiv l^{-1} pH^{-1}) the post-exercise depressions in haemolymph pH were very similar; individual variations in lactate and P_{CO_2} increase thus obscured any minimizing influence that a high buffering capacity might have had. In the female crabs substantially smaller acid-base disturbances were observed (Fig. 3). This was, however, correlated with substantially lower haemolymph lactate levels rather than buffer capacity (buffer capacities 3.9 and 5.5 m-equiv l^{-1} pH^{-1}). The lower levels of lactate in these animals may be due to an increased aerobic metabolic capacity but a more likely explanation is that the procedures used to provoke activity may have had a generally less stimulating effect on metabolism in these smaller animals.

The nature of the acid-base disturbance provoked by strenuous exercise has not previously been studied in detail in crustaceans and thus no direct comparisons are possible. However the response of the crab is quantitatively similar to the response of the dogfish *Scyliorhinus canicula* (Piiper *et al.* 1972). Peak lactate levels are similar in these species (18.0 ± 4.0, dogfish and 11.5 ± 2.4 m-equiv l^{-1}, crab) and lactic acid is the principal source of the post-exercise depression in pH. Furthermore, in both species marked discrepancies between buffered metabolic acid and measured lactate levels in the blood (or haemolymph) are apparent in the post-exercise recovery period. In the crab the occurrence of a more gradual increase and decrease in $\Delta H^{+}m$ than the corresponding changes in lactate levels (Fig. 5) suggests that the H^{+} ions produced in

the tissues in equivalent amounts with lactate diffuse into the haemolymph more slowly than lactate anions. Piiper *et al.* (1972) similarly attributed this discrepancy to the intracellular retention of H^+ ions since they found that the H^+ ion elimination rate in the dogfish was too small to account for the differences between lactate and H^+ ion levels in the blood. The initial changes in ΔH^+m are similar in the dogfish and the crab but in the dogfish resting levels are restored by the same time ($\sim$ 24 h) as blood lactate levels whereas in the crab ΔH^+m was still significantly elevated 48 h after lactate had returned to normal (Fig. 5). The persistent metabolic acidosis in the crab is somewhat surprising since at least two processes may be operating in the recovery period to remove H^+ ions from the haemolymph: branchial excretion of H^+ ions (see below) and uptake of H^+ ions by those tissues wherein lactic acid is metabolized. Thus it may be that the acid-base disturbance apparent in the later stages of the recovery period is not related to the initial exercise-induced lactacidosis but rather to experimental disturbances such as repetitive haemolymph sampling. These disturbances may then either result in acid metabolites other than lactic acid being added to the haemolymph or may stimulate branchial or renal losses of bicarbonate.

Although the post-exercise increase in haemolymph lactate concentration is more rapid than the increase in H^+ ion produced with lactate the time course of lactate release and removal from haemolymph while similar to that seen in the dogfish (Piiper *et al.* 1972) and the flounder *Platichthys stellatus* (Wood *et al.* 1977) is much slower than in man. In the crab, peak lactate concentrations are not reached for 1–2 h following exercise (Fig. 3) whereas in man, similar quantities of lactate are produced but peak lactate concentrations in the blood are reached a few minutes after exercise and decrease to a resting value with a half-time of about 15 min (Margaria *et al.* 1963). Furthermore, in man the increase in blood lactate levels is correlated with the decrease in blood bicarbonate indicating that hydrogen ions move out of the tissues in equivalent amounts with lactate (Keul, Doll & Keppler, 1972). The slower outward efflux of lactate in the crab may be attributable to the effect of low body temperatures on the rate of diffusion although Mainwood & Worsley-Brown (1975) have shown that extracellular buffer concentration has an important influence on the efflux rates of both lactate and hydrogen ions. When isolated frog sartorius muscle superfused with a high concentration (25 mM) buffer was stimulated electrically, lactate efflux rate was high and lactate and hydrogen ion effluxes were closely coupled. At low external buffer concentrations (1 mM) the rate of lactate efflux was considerably reduced and 80–90% of the efflux was in the form of lactate ions only, H^+ ion being retained intracellularly. Since the buffering power (representative of buffer concentration) is substantially lower in crab haemolymph (2–8 m-equiv l^{-1} pH^{-1}) in comparison to man (27 m-equiv l^{-1} pH^{-1}; Woodbury, 1974) this may partially explain the difference in the responses.

The slow disappearance of lactate from the haemolymph in the crab may be similarly attributable to low body temperatures which would affect its rate of metabolic consumption either by conversion to carbohydrate or by oxidation to CO_2 and H_2O. The hepatopancreas may also have a limited capacity to metabolize lactate (Phillips *et al.* 1977) although this may be augmented by the gills which have a pronounced gluconeogenic capacity in crustaceans (Thabrew, Poats & Munday, 1971). Recent evidence suggests that lactate is not removed from the haemolymph by

excretion. C. R. Bridges & A. R. Brand (personal communication) were unable to detect the presence of excreted lactate in experiments on several species of decapod crustaceans recovering from a severe hypoxia induced lactacidosis.

The calculations reported above indicate that over the first hour following exercise a greater quantity of both metabolic (ΔH^+m) and respiratory acid (ΔH^+c) is present in pre-branchial haemolymph compared to post-branchial haemolymph. This reduction of respiratory acid levels when the haemolymph transits the gills can be attributed to diffusional losses of CO_2 but the reduction in metabolic acid levels suggests that a direct efflux of H^+ ions or a direct influx of HCO_3^- ion (its functional equivalent) occurs across the gill epithelium. As far as we are aware this constitutes the first direct evidence for H^+ ion elimination by this route in a marine crustacean. In the freshwater crayfish *Astacus leptodacylus* (Ehrenfeld, 1974) and in the marine crab *Callinectes sapidus* adapted to freshwater (Cameron, 1978) H^+ ion elimination or HCO_3^- absorption of the gills is thought to occur via electroneutral exchanges for Na^+ and Cl^- ions respectively. Whether such exchanges also operate in *C. magister* is unknown but would seem likely given the ready availability of the appropriate counter-ions in sea water. The existence of a persistent metabolic acidosis in the crab however suggests that the capacity of such mechanisms to precisely regulate haemolymph acid-base balance is limited. Clearly branchial fluxes of ions at rest and during acidotic conditions must be measured and quantified before this question can be resolved.

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