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ANALYSIS OF HAEMOLYMPH AND MUSCLE ACID–BASE STATUS DURING AERIAL EXPOSURE IN THE CRAYFISH *AUSTROPOTAMOBIUS PALLIPES*

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SUMMARY

1. Exposure of the crayfish *Austropotamobius pallipes* to air resulted in an acidosis in the postbranchial haemolymph (pHa) and the abdominal muscle. The haemolymph acidosis was subsequently compensated and, after 24 h in air, pHa had returned to the settled, submerged value. The intracellular acidosis remained uncompensated throughout the period of aerial exposure.

2. When crayfish were first removed into air, lactate concentrations in the haemolymph and abdominal muscle increased substantially. After 24 h in air lactate concentrations in both compartments had returned towards submerged levels. Possibilities for the fate of lactate are discussed.

3. Re-analysis of haemolymph acid–base data for crayfish exposed to air (Taylor & Wheatly, 1981) revealed discrepancies between observed and expected base excess. Initially these may arise from exchanges of H^+ or HCO_3^- with other compartments. During long-term air exposure, the removal of lactate from the haemolymph and an independent accumulation of base, probably from the mobilization of an internal source of bicarbonate buffer, result in the observed pH compensation.

4. Determination of base excess for the changes in abdominal muscle acid–base status after 3 h of exposure to air corroborated the results of the haemolymph analysis, suggesting a retention of H^+ despite the efflux of lactate.

INTRODUCTION

The extracellular pH (pHe) of aquatic, gill-breathing animals has been shown to vary considerably at constant temperature, under the influence of external factors, such as the partial pressure of oxygen (P_{O_2}) (Dejours, Truchot, Armand & Beekenkamp, 1974; Dejours & Beekenkamp, 1977; Dejours & Armand, 1980; Wheatly & Taylor, 1981; Wilkes & McMahon, 1982), the partial pressure of carbon dioxide (P_{CO_2}) (Cameron, 1978) and chloride concentration (Dejours, Armand &

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Beekenkamp, 1982). Since pHe is affected by changes in a variety of environmental factors it is possible that regulation of intracellular pH (pHi) is of primary importance for homeostasis. *In vivo* studies have shown the precedence of pHi regulation over pHe in most tissues of aquatic animals (Heisler, 1982; Heisler, Forcht, Ultsch & Anderson, 1982; Gaillard & Malan, 1983).

The respiratory consequences of emersion have been studied in a number of amphibious crustaceans. Shore crabs, which are frequently exposed to air, have been the subject of the majority of these studies (Truchot, 1975; Taylor & Butler, 1978; Wheatly & Taylor, 1979; DeFur, McMahon & Booth, 1983; DeFur & McMahon, 1984*a*,*b*; Innes *et al.* 1986). However, the response to emersion has also been studied in freshwater crayfish which, although primarily aquatic animals and rarely exposed to the aerial environment, will voluntarily leave hypoxic water to breathe air (Taylor & Wheatly, 1980, 1981; McMahon & Wilkes, 1983). Although the changes in haemolymph acid–base status resulting from emersion have been described in both shore crabs and crayfish, the effects of aerial exposure on intracellular acid–base status have not previously been examined.

The progressive changes in haemolymph acid–base status which occur in the crayfish *Austropotamobius pallipes* during aerial exposure have been described in detail by Taylor & Wheatly (1980, 1981). Briefly, an initial acidosis in the haemolymph, of both respiratory and metabolic origin, occurs on removal into air. During long-term exposure the acidosis is progressively compensated by the elevation of bicarbonate buffer levels, $[HCO_3^-]$, and a reduction in circulating lactate concentrations until, after 24 h in air, haemolymph pH (pHe) returns towards submerged values.

In this study, results from an investigation into changes in abdominal muscle pH and lactate content during aerial exposure of crayfish are reported. These data prompted a re-analysis of the quantitative relationships between changes in pH and lactate concentration in the haemolymph following that of Taylor & Wheatly (1981) and a similar analysis of muscle acid–base status.

MATERIALS AND METHODS

All crayfish (*Austropotamobius pallipes*) used in the experimental procedures outlined were collected and maintained as described by Taylor & Wheatly (1980).

Intracellular pH

Measurements were taken from 18 crayfish of either sex and of mean mass 26·3 g (range 13·2–51·5 g). At least 1 week prior to the experiments animals were removed from the holding tanks and kept in well-aerated, dechlorinated Birmingham tapwater at 15 ± 1 °C. Intracellular pH was determined on submerged crayfish and on crayfish exposed to air for 3 or 24 h. Both water and air temperatures were 15 ± 0·5 °C during experimental procedures.

For the determination of pHi animals were injected with 50 μl of crayfish saline containing 60 μCi ml^{-1} of [^{3}H]inulin and 20 μCi ml^{-1} of 5,5-dimethyl[2-^{14}C]-oxazolidine-2,4-dione (^{14}C DMO) (Radiochemical Centre, Amersham). The injections were made through a hole previously drilled in the dorsal carapace, above the heart and covered by a rubber septum. The needle was inserted into the pericardial space but not far enough to damage the heart. After injection the animals were held in individual tanks containing either air or aerated water until sampling.

Animals were sampled 3–4 h after injection. This period appeared adequate to allow equilibrium distribution of DMO to be achieved since steady-state pHi values were obtained. Postbranchial haemolymph, sampled anaerobically using a glass syringe, was taken from the pericardial space *via* the predrilled hole. Haemolymph pH was determined using a capillary microelectrode (Radiometer, E5021a) thermostatted to 15 ± 0·3 °C and calibrated immediately before each determination. Subsamples of haemolymph were pipetted onto glass trays for oxidation and analysis of isotope content. The animals were then killed by destruction of the cerebral ganglia and the abdominal muscles exposed and carefully removed. The time between haemolymph sampling and dissection of the abdominal muscle was approximately 2 min. Each muscle sample was divided into two subsamples. These were weighed then dried at 100 °C to constant mass and tissue water content was calculated. The overestimation of water content due to the loss of lipids at this temperature would be negligible since the lipid content of crayfish muscles is less than 1 % of fresh mass (O'Connor & Gilbert, 1969).

Haemolymph and tissue samples were oxidized in a biological oxidizer (R. J. Harvey Instrument Corporation, OX400). Samples, trapped after combustion, were counted in a liquid scintillation counter (Beckman, LS1800). The inulin space of each sample was calculated and taken as an estimate of tissue extracellular fluid volume (ECFV). Intracellular pH was calculated from the distribution of DMO using the equation of Waddell & Butler (1959) and a pK value of 6·31 for DMO (Roos, 1978).

Haemolymph and tissue lactate concentrations

L-lactate determinations were made on haemolymph and abdominal muscle from 17 crayfish, either submerged in water or exposed in air for 3 or 24 h at 15 °C.

Haemolymph samples were taken from the arthrodial membrane at the base of a walking leg using a cooled (5 °C) glass syringe. Subsamples were immediately pipetted into cold 8 % perchloric acid. Animals were then killed and the abdominal flexor and extensor muscles dissected out. A sample of approximately 0·3 g was removed, blotted on filter paper, then weighed. Each sample was homogenized in cold 8 % perchloric acid using a mortar and pestle. After homogenization the mortar and pestle were carefully rinsed with perchloric acid which was added to the homogenate for the determination of L-lactate concentration.

All samples were centrifuged at 3000 rev. min^{-1} for 10 min. The supernatant was decanted and 0·2 ml used for the determination of lactate concentration by the enzymatic reduction of NAD (Sigma Technical Bulletin, 826-UV).

Analysis of haemolymph acid–base variables

The relationships between changes in pH and lactate concentration in the haemolymph of crayfish exposed to air were investigated using an analysis similar to that described by Davenport (1969) and Wood, McMahon & McDonald (1977). This enabled the base excess/deficit and the relative contributions of the respiratory and metabolic components of pH changes (calculated as percentage of changes in $[H^+]$) to be estimated.

Data from Taylor & Wheatly (1981) were used for the analysis of haemolymph acid–base status. The *in vitro* buffer value for *Austropotamobius* haemolymph used throughout the calculations was 13 mequiv l^{-1} pH $unit^{-1}$ (Taylor & Wheatly, 1981). Although evaporative water losses during prolonged aerial exposure might lead to a gradual increase in the buffer value, any resulting errors in the estimate of expected base excess are likely to be small since haemolymph osmolarity increases by only 3 % over 24 h of exposure (Taylor, Tyler-Jones & Wheatly, 1987). The values of α and pK for CO_2 used were 0·43 and 6·23, respectively (E. W. Taylor & M. G. Wheatly, unpublished data).

The *in vivo* acidosis in the haemolymph after 1·75 h in air had both respiratory and metabolic components (see Results). Since these two components probably changed simultaneously it is not possible to determine the instantaneous contribution of either to the observed progressive acidosis. However, by taking the two extreme situations, limits can be defined for their relative contributions (refer to Fig. 1). If CO_2 accumulated at a constant acid load, then the haemolymph would titrate along the buffer line to the final P_{CO_2} isopleth. The contribution of CO_2 to the acidosis would thus be measured as the horizontal distance (x) from this point of intersection to the original pH. Alternatively, if lactic acid had accumulated at constant P_{CO_2} the haemolymph would titrate along the original P_{CO_2} isopleth to the point of intersection with a buffer line drawn through point 1·75hA. In this case the contribution of CO_2 would be given by the horizontal distance (y) from this point of intersection to the final pH.

Results are expressed as mean ± 1 S.E.M. with the number of observations given in parentheses. Differences between means were subjected to Student's *t*-test and significance assigned at the 95 % level.

RESULTS

Haemolymph and intracellular pH

Haemolymph pH measured in submerged animals was similar to the value found by Taylor & Wheatly (1981) for settled, submerged crayfish indicating that the animals had recovered from the stress induced by injection (Table 1). Three hours of aerial exposure resulted in a significant acidosis in the prebranchial haemolymph. After 24 h in air pHa had recovered to a value not significantly different from the settled, submerged value but significantly higher than the 3 h value.

Table 1. *Values for postbranchial haemolymph and abdominal muscle pH (pHa and pHi, respectively) and lactate concentration, expressed as mg g^{-1} fresh mass of muscle sample, for crayfish submerged in normoxic water and exposed in air*

	Present study				Taylor & Wheatly (1981)	
	Abdominal muscle		Haemolymph		Haemolymph	
	pHi	Lactate (mg g^{-1} fresh mass)	pHa	Lactate (mmol l^{-1})	pHa	Lactate (mmol l^{-1})
Submerged	7·321 ± 0·019 (6)	0·15 ± 0·01 (6)	7·841 ± 0·022 (6)	0·27 ± 0·05 (6)	7·896 ± 0·024 (9)	0·55 ± 0·15 (11)
1–2 h in air	—	—	—	—	7·457 ± 0·038 (7)	8·28 ± 0·86 (7)
3 h in air	7·122 ± 0·019 (6)	0·58 ± 0·12 (6)	7·535 ± 0·054 (6)	2·69 ± 0·51 (6)	—	—
24 h in air	7·140 ± 0·045 (6)	0·23 ± 0·05 (6)	7·827 ± 0·056 (6)	0·53 ± 0·21 (5)	7·789 ± 0·016 (6)	0·57 ± 0·16 (6)

Corresponding data from Taylor & Wheatly (1981) are also tabulated for comparison.

Intracellular pH of the abdominal muscles in settled, submerged animals was 7·321 ± 0·019 (6), about 0·52 pH units below pHa. After 3 h of aerial exposure pHi showed a significant acidosis which remained unchanged over the ensuing 21 h of exposure (Table 1).

L-lactate concentrations

Haemolymph lactate showed a large and significant increase during the first 3 h of aerial exposure, increasing by 10 times from the submerged value. Following 24 h of exposure to air haemolymph [lactate] had returned towards the submerged level. This value was not significantly different from the submerged level, but represented a significant recovery from the 3 h value (Table 1).

Lactate levels in the abdominal muscle increased significantly during the first 3 h of aerial exposure (Table 1). Over the ensuing 24 h in air tissue [lactate] decreased to a value which was not significantly different from the submerged level but was significantly lower than the 3 h value. Correcting for lactate in the extracellular fluid within the muscle, these data yield values for intracellular lactate concentration of 2·80 mmol l^{-1} in submerged crayfish and 9·95 and 3·94 mmol l^{-1} in animals after 3 and 24 h exposure in air, respectively.

Values for pHa, pHi and haemolymph and tissue lactate are given in Table 1 together with the relevant data from Taylor & Wheatly (1981) for comparison. The two sets of data are similar in that the crayfish experienced an initial acidosis after 1·75–3 h in air with elevated haemolymph lactate levels. After 24 h in air haemolymph pH and lactate concentrations had returned towards submerged levels.

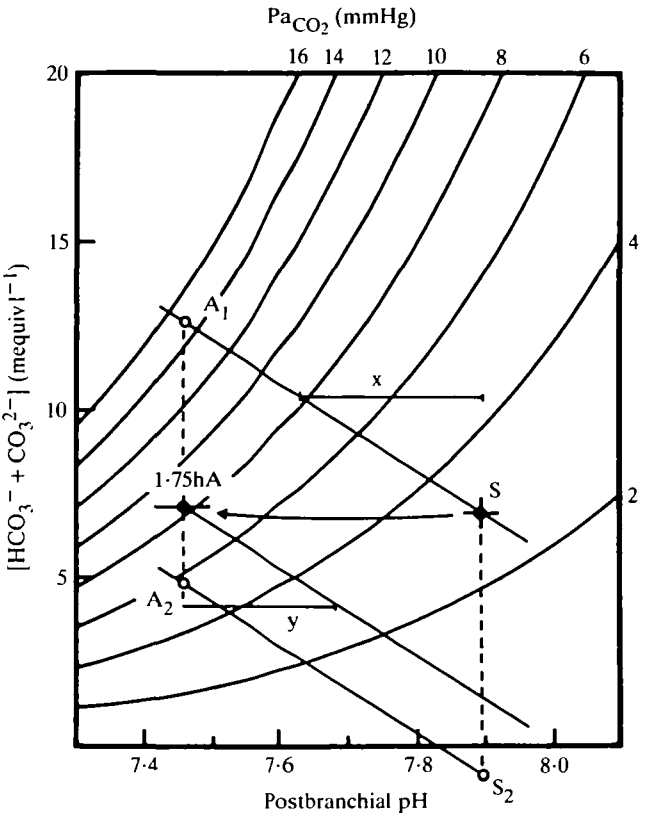

Fig. 1. Variation in mean (± S.E.M.) pH and $[HCO_3^-]$ in the postbranchial haemolymph of crayfish settled in normoxic water (S) and after 1·75 h of exposure in air (1·75hA). The distance $S–S_2$ is the change in expected base excess given by the measured change in lactate concentration expressed as $HCO_3^- + CO_3^{2-}$ units. Further details of the analysis are given in the text. Data from Taylor & Wheatly (1981).

Analysis of acid–base data

Haemolymph acid–base data for crayfish submerged in water and exposed in air for 1·75 h are illustrated as a pH–bicarbonate diagram in Fig. 1. The observed base deficit (vertical distance A1–1·75hA) is equivalent to 5·51 mequiv l^{-1} of $[HCO_3^- + CO_3^{2-}]$. The measured increase in lactate concentration (expected base deficit) was 7·73 mequiv l^{-1} of $[HCO_3^- + CO_3^{2-}]$ equivalents (vertical distance $S–S_2$ on Fig. 1) giving a discrepancy between the two values of 2·22 mequiv l^{-1} of $[HCO_3^- + CO_3^{2-}]$ indicated by the distance 1·75hA–A_2 on Fig. 1.

The accumulation of CO_2 accounts for between 50 and 60 % of the acidosis after 1·75 h in air (lines x or y on Fig. 1 as described in Materials and Methods), a substantially greater contribution than the value of 30 % estimated from these data by Taylor & Wheatly (1981). The accumulation of metabolic acid accounts for the remaining 40–50 % of the acidosis.

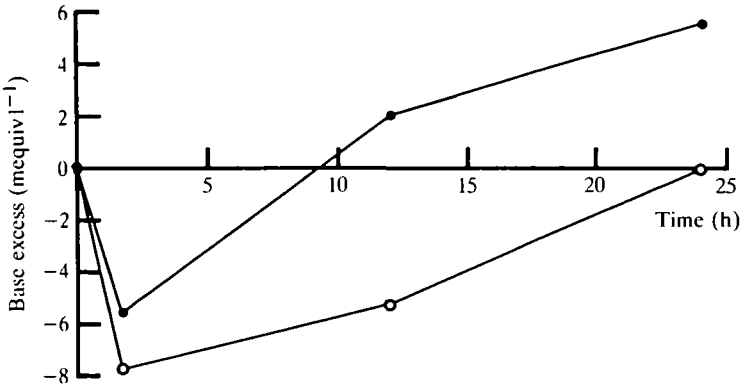

Fig. 2. Observed and expected base excess in the postbranchial haemolymph of crayfish settled in normoxic water and during exposure to air calculated from the data of Taylor & Wheatly (1981). The initial filled symbol represents the value for submerged animals. The filled symbols joined by continuous lines are the values for observed base excess for animals exposed to air for 3, 12 and 24 h. The open symbols are the expected base excess (lactate concentration) of exposed crayfish.

The changes in observed and expected base excess in the haemolymph during aerial exposure are summarized in Fig. 2. The observed base deficit decreased during aerial exposure from 5·51 mequiv l^{-1} after 1·75 h in air and subsequently gave rise to a base excess of increasing magnitude. The discrepancy between the observed and expected base excess values increased between 1·75 and 12 h of exposure and thereafter remained elevated.

DISCUSSION

The decrease in intracellular lactate levels which occurs in the abdominal muscles of crayfish following the high value 3 h after exposure to air suggests that the removal of lactate from the haemolymph during long-term exposure to air is not due to its accumulation in these muscles. The possibility that lactate is accumulated in other tissues cannot, however, be excluded. There is no evidence for the reoxidation of lactate during aerial exposure since oxygen consumption in air is maintained at a rate similar to that measured in settled, submerged crayfish (Taylor & Wheatly, 1980, 1981) and crustaceans appear to be unable to excrete lactate (Bridges & Brand, 1980).

On resubmersion in water after 3 or 24 h of aerial exposure a transient, but significant, increase in the rate of oxygen consumption was reported by Taylor & Wheatly (1980, 1981). After 24 h of exposure, this increase coincided with the reappearance of elevated haemolymph lactate levels. Taylor & Wheatly (1981) suggested that the transient elevation of oxygen consumption resulted from disturbance of the animals and that there was no progressive accumulation of an oxygen debt during aerial exposure. However, it is possible that the increased oxygen consumption on resubmersion represents the repayment of an oxygen debt incurred on initial removal from water when haemolymph oxygen content is significantly

reduced, possibly due to collapse of the gills. The lactate removed from the haemolymph during prolonged aerial exposure in the crayfish may be sequestered in tissues other than the abdominal muscles. On resubmersion it may be redistributed (resulting in a transient elevation of haemolymph lactate level) for reoxidation, as indicated by an increase in the rate of oxygen consumption.

The data for abdominal muscle pHi in *Austropotamobius pallipes* compare favourably with available *in vivo* data for other aquatic animals both in the pHe−pHi difference (e.g. Gaillard & Malan, 1983, 1985; Hōbe, Wood & Wheatly, 1984; Cameron, 1980; Heisler, Weitz & Weitz, 1976) and in the $\Delta pHi/\Delta pHe$ ratio during acidosis (Cameron, 1980; Gaillard & Malan, 1983).

After 24 h of exposure the acidosis observed in the abdominal muscles remained uncompensated and the pHe−pHi difference had increased to 0·69 pH units due to the recovery of pHa. Direct measurements of pHi in the abdominal muscle of restrained crayfish by E. W. Taylor & R. C. Thomas (unpublished data) using pH-sensitive microelectrodes, confirmed that pHi remained uncompensated in air. In aquatic and bimodally breathing animals acid–base regulation is normally biased towards the maintenance of pHi rather than pHe (Heisler, 1982; Heisler *et al.* 1982; Hōbe *et al.* 1984). This precedence of pHi regulation occurs in crayfish heart and nervous tissue (Gaillard & Malan, 1983, 1985) but appears not to occur in the abdominal muscles since pHi disturbances in these muscles during hypoxia and hypercapnia (Gaillard & Malan, 1983), a temperature-induced alkalosis (Gaillard & Malan, 1985) and aerial exposure (this study) remain uncompensated.

Gaillard & Malan (1985) suggested that predominantly glycolytic muscle cells, like the crayfish abdominal muscles, may represent a special case for pHi regulation. The slow, ionic mechanism for pH regulation in these tissues (Rodeau, 1984) results in a non-equilibrium value of pHi and this may represent a second line of defence during prolonged acid loads. This behaviour of the abdominal muscles may enable other tissues requiring greater pH stability, such as heart muscle and nerve cord, to compensate for pHi disturbances as the potential haemolymph acid load is reduced by the retention of acid in the muscles (Burton, 1980). Thus, the lack of compensation in abdominal muscles may represent an example of 'altruistic' behaviour, as shown by erythrocytes and some skeletal muscles in vertebrates (Burton, 1978).

The pre-eminent determinants of pH in most biological systems are the partial pressure of carbon dioxide (P_{CO_2}) and the strong ion difference (SID) (Stewart, 1978) of which lactate, acting as a strong acid in the physiological range of pH values, is a component. The observed haemolymph base deficit after 1·75 h in air indicates an excess of H^+ associated with a change in SID, due largely to the accumulation of lactate. Although the metabolic accumulation of acid accounts for 40–50 % of the acidosis, the specific contribution of lactate accumulation cannot be determined since it is only one component of the change in SID and therefore not directly related to pH.

The discrepancy between the observed and expected base deficit after 1–2 h of exposure implies a deficiency of H^+ relative to the increase in lactate ions.

Compensatory changes in the other components of the SID must also occur which give rise to either a loss of H^+ from, or an accumulation of excess HCO_3^- buffer in, the haemolymph (Wood *et al.* 1977). After 2 h in air, haemolymph calcium levels are unchanged from the submerged value (Morris, Tyler-Jones, Bridges & Taylor, 1986) suggesting that the dissolution of carapace carbonates as a source of bicarbonate buffer is insubstantial. Branchial uptake of HCO_3^- or excretion of H^+ *via* ion exchange mechanisms probably cannot account for the discrepancy since, during aerial exposure, these exchanges would be severely limited by the loss of contact with water. The discrepancy may therefore involve either the loss of HCO_3^- from, or, equivalently, the accumulation of H^+ within, other tissues.

The increase in observed base excess during long-term exposure can be attributed partly to the reduction in lactate levels. However, the increase in the difference between the observed and expected base excess indicates an independent accumulation of base, probably due to the mobilization of an internal source of the fixed base, $CaCO_3$, as proposed by Taylor *et al.* (1987). These two mechanisms together effect a virtually complete compensation for the acidosis despite the maintained hypercapnia. The mobilization of bicarbonate in the carapace or other internal sources as a mechanism for providing HCO_3^- buffer also occurs in other crustacean species (DeFur, Wilkes & McMahon, 1980; Cameron, 1981; Henry, Kormanik, Smatresk & Cameron, 1981; Wood & Randall, 1981*b*).

Intracellular acid–base status after 3 h of exposure was analysed by the method described for the haemolymph and using the derived value for intracellular lactate concentration as an estimate of expected base excess. For the analysis a number of assumptions were made. The values of α and pK for CO_2 used were the same as those for the analysis of haemolymph variables. The intracellular buffer value had not been estimated and the value used, 46 mequiv l^{-1} pH $unit^{-1}$, was determined by England & Baldwin (1983) for the tail muscle of the Australian crayfish, *Cherax destructor*. Finally, the P_{CO_2} values used for the analysis of intracellular acid–base status were taken from Taylor & Wheatly (1981) for prebranchial haemolymph (Pv_{CO_2}), on the assumption that intracellular P_{CO_2} is unlikely to differ significantly from Pv_{CO_2} owing to the high diffusive conductance of CO_2 through tissues relative to that of O_2 (Dejours, 1975).

This analysis, illustrated in Fig. 3, indicates that after 3 h in air the acidosis was due predominantly (75–78 %) to the accumulation of metabolic acid. The increase in lactate concentration (expected base deficit) accounts for 89 % (the distance A_1–A_2 on Fig. 3) of this metabolic acidosis (observed base deficit). The remaining 11 % of the metabolic acidosis (3·0hA to A_2 on Fig. 3) was due to an accumulation of H^+ independent of the increase in lactate concentration. Only 22–25 % of the acidosis was attributable to the increase in P_{CO_2} from 3·5 to 8·4 mmHg (1 mmHg = 133·3 Pa) (respiratory acidosis).

The intracellular base deficit after 3 h in air suggests an accumulation of H^+ associated with changes in the SID, due largely (89 %) to the increase in lactate levels. The discrepancy between observed and expected base deficits could be due to

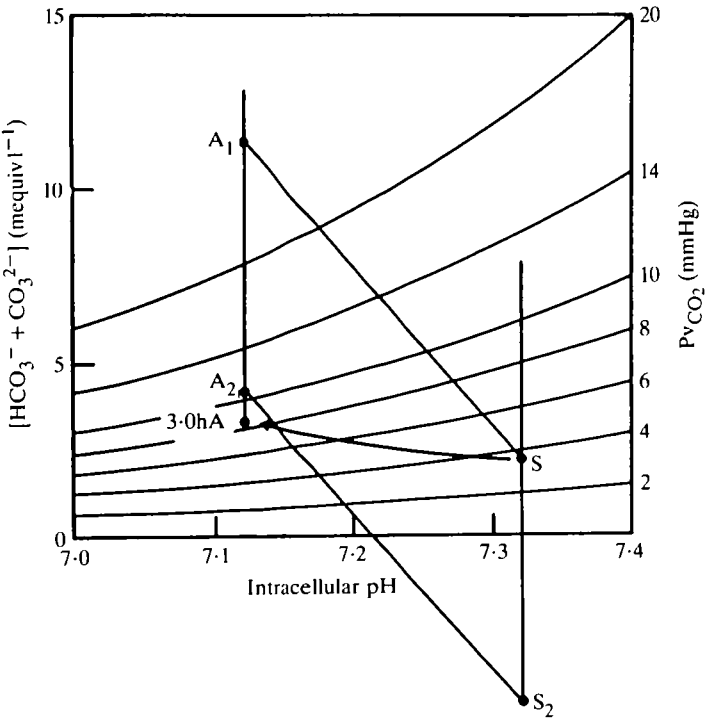

Fig. 3. Mean changes in intracellular pH (pHi) and bicarbonate levels for the abdominal muscles of crayfish settled in normoxic water (S) and after 3 h of exposure to air (3·0hA). Further details are given in the text. As in Fig. 1 the distance $S–S_2$ indicates the expected base excess.

errors in any of the assumptions made in the analysis. However, if the assumed values are approximately correct, possible sources for the apparent discrepancy are either the production of acids other than lactate during the period of anaerobiosis or the accumulation of H^+ in, or loss of HCO_3^- from, the intracellular compartment (Wood *et al.* 1977). The first possibility can be discounted as lactate is the sole important end-product of anaerobic metabolism in decapod crustaceans (Trausch, 1976; Gäde, 1984). The selective loss of HCO_3^- or retention of H^+ in the muscle would substantiate the excess of HCO_3^- or deficit of H^+ in the haemolymph after 1·75 h in air.

Although lactate level increases in the abdominal muscles on initial exposure to air, it does not necessarily infer an accompanying and equivalent metabolic acidosis since lactate ions and H^+ are separable and only lactate ions were measured in the assay used. Thus the efflux of lactate from the muscles during the period of anaerobiosis when crayfish are first removed from water may not be accompanied by an equivalent efflux of H^+, and a partial retention of H^+ in the muscles may occur. An intracellular retention of H^+ during recovery from exercise has been postulated in the elasmobranch *Scyliorhinus stellaris* by Piiper, Meyer & Drees (1972), and in the crabs

Cancer magister and *Birgus latro* by McDonald, McMahon & Wood (1979) and Smatresk & Cameron (1981), respectively.

The principle mechanisms employed for acid–base regulation by aquatic crustaceans are the branchial ion exchanges, $Na^+/H^+(NH_4^+)$ and Cl^-/HCO_3^- (Cameron, 1978; Truchot, 1979; Dejours *et al.* 1982). These mechanisms are retained in the gills of terrestrial crustaceans (Towle, 1981) and may be used for acid–base regulation by exchanges with pools of water carried in the branchial chambers (Wood & Randall, 1981*a*). However, these mechanisms may be limited in efficacy or precluded by the loss of gill contact with water. Alternative mechanisms for achieving acid–base balance, including respiratory control (McMahon & Burggren, 1981; Smatresk & Cameron, 1981; Wheatly, Burggren & McMahon, 1984), dissolution of carapace carbonates as a source of bicarbonate (Henry *et al.* 1981; Wood & Randall, 1981*b*; DeFur & McMahon, 1984*b*) and intracellular/extracellular Na^+/H^+ and Cl^-/HCO_3^- exchanges (Cameron, 1981), can be used by terrestrial crabs when branchial exchanges are ineffective.

In crayfish tissues the principal intracellular/extracellular ion exchanges are $Na^+/H^+/HCO_3^-/Cl^-$ and Na^+/H^+ (Thomas, 1984; Moser, 1985; Galler & Moser, 1986). Crayfish exposed to air lose gill contact with appreciable amounts of water and consequently lose their ability to regulate acid–base balance *via* branchial ion exchanges. To restore normal acid–base status following the initial acidosis resulting from aerial exposure, they appear to resort to the mobilization of internal buffer and to internal ion exchanges, possibly involving the sequestration of H^+ in muscle, mechanisms also available to terrestrial crustaceans.

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