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THE EFFECT OF LONG-TERM AERIAL EXPOSURE ON HEART RATE, VENTILATION, RESPIRATORY GAS EXCHANGE AND ACID-BASE STATUS IN THE CRAYFISH *AUSTROPOTAMOBIUS PALLIPES*

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SUMMARY

1. When first removed into air, crayfish showed transient increases in heart rate (f_H) and scaphognathite rate (f_R) which rapidly recovered to submerged levels and were unchanged for 24 h. The rate of O_2 consumption ($\dot{M}_{O_2}$) increased from an initially low level and was then maintained for 24 h in air at the same level as in settled submerged animals.

2. There was an initial acidosis in the haemolymph which was both respiratory and metabolic due to the accumulation of CO_2 and lactate. Progressive compensation by elevation of the levels of bicarbonate buffer in the haemolymph and reduction of circulating lactate levels returned pH towards submerged levels after 24 h in air.

3. Exposure to air resulted in a marked internal hypoxia with haemolymph O_2 tensions, both postbranchial P_{a,O_2} and prebranchial P_{v,O_2} remaining low throughout the period of exposure. The oxygen content of the haemolymph was initially reduced, with $a-v_{O_2}$ content difference close to zero. Within 24 h both C_{a,O_2} and C_{v,O_2} had returned towards their levels in submerged animals. These changes are explained by the Bohr shift on the haemocyanin consequent upon the measured pH changes.

4. After 48 h in air, $\dot{M}_{O_2}$ and f_H were significantly reduced and ventilation became intermittent. There was a slight secondary acidosis, increase in lactic acid levels and reduction in $a-v_{O_2}$ content difference in the haemolymph.

5. When crayfish were returned to water after 24 h in air, $\dot{M}_{O_2}$, f_H and f_R were initially elevated by disturbance and there was a period of hyperventilation. In the haemolymph there was an initial slight alkalosis, and an increase in C_{a,O_2} and lactic acid. All variables returned to their settled submerged levels within 8 h.

INTRODUCTION

There are many important differences in the patterns of respiratory gas exchange and the maintenance of acid–base balance in the body fluids, between water- and air-breathing animals (Dejours, 1975, 1978). Put simply, in air there is a ready supply of oxygen but the elimination of carbon dioxide can present problems, whereas in water the availability of O_2 is limited in a number of ways, whilst elimination of

CO_2 in solution is no problem. As a consequence, relatively few animals have developed the capability to respire equally well in both media. Such bimodal respiration is shown by air-breathing fishes (Johansen, 1970) and amphibians (Gottlieb & Jackson, 1976) amongst the vertebrates, and by a few molluscan and crustacean species. Some bimodal breathers may exploit the availability of O_2 in the aerial environment whilst retaining the aquatic route for CO_2 exchange by aerating the water surrounding their gills or using the body surface to exchange CO_2. This behaviour was termed 'Notatmung' (Carter, 1931) and forms the basis of the 'emersion' response in the shore crab *Carcinus maenas* (Taylor, Butler & Sherlock, 1973; Wheatly & Taylor, 1979).

The class Crustacea includes many species which are able to survive in air, either having ventured on to land permanently such as the isopods (Edney, 1960) and land crabs (e.g. Cameron & Mecklenberg, 1973) or being exposed for short periods only such as the shore crab (Taylor & Butler, 1978; Taylor & Wheatly, 1979).

In a recent study, Taylor & Wheatly (1980) demonstrated that the freshwater crayfish *Austropotamobius pallipes* migrated from hypoxic water, with a mean P_{O_2} of 42 mmHg at 15 °C, into air where after 3 h it experienced a marked internal hypoxia and an acidosis. Rate of oxygen consumption was however maintained at the settled submerged level, apparently by utilization of a venous reserve of oxygen. Despite this maintained $\dot{M}_{O_2}$, the animals accumulated lactate but did not appear to repay an oxygen debt on replacement in water, as the only obvious change in respiratory behaviour following 3 h aerial exposure was a brief period of hyperventilation.

Further observation has revealed that *Austropotamobius* can survive for prolonged periods in damp air (R.H. 70–80%) at 15 °C, remaining fairly active for up to 24 h but becoming immobile at 48 h. By 72 h most animals appear moribund though individuals can survive in air for as long as 6 days.

During longer periods of aerial exposure the physiological problems for a primarily aquatic, gill-breathing animal are likely to be cumulative. The present study describes the progressive changes in heart rate, ventilation rate, respiratory gas exchange and acid–base balance in the crayfish within the first hour of exposure to air and following 12 h, 24 h and 48 h in air, and compares them with the settled submerged values prior to aerial exposure and to the changes following replacement in water after 24 h in air.

MATERIALS AND METHODS

The present report contains observations on 81 *Austropotamobius pallipes* (Lereboullet), the common British freshwater crayfish, of either sex and of mass between 18 and 79 g. The animals were from an indigenous population in Chasewater, Cannock Chase, Staffordshire (O.S. grid reference 120/072-039, 1979). Before experiments, the animals were kept under the conditions described by Taylor & Wheatly (1980). All the experiments described in this investigation were conducted at 15 ± 1 °C either in water or in air with a relative humidity of 70–80%. Each animal was used once only.

All the techniques employed in this study are fairly standard and their use in our laboratory has been described in earlier publications. Briefly, rate of oxygen consumption ($\dot{M}_{O_2}$) in water was measured in a continuous flow respirometer (Taylor, Butler & Al-Wassia, 1977*a*) with water samples analysed using a P_{O_2} electrod

nnected to a blood gas analyser (Radiometer PHM 71). The same respirometer was used to measure $\dot{M}_{O_2}$ in air (Taylor & Butler, 1978) but was connected to a paramagnetic oxygen analyser (Servomex). Heart rate (f_H) was measured as an ECG and respiratory frequency (f_R) as the fluctuations in hydrostatic pressure generated by the scaphognathite in the branchial chambers surrounding the gills, which were measured using a pressure transducer (Elcomatic). These methods are eludicated in Taylor *et al.* (1973), Taylor *et al.* (1977*a*) and Butler, Taylor & McMahon (1978).

The levels of the respiratory gases and the acid–base status were measured on prebranchial haemolymph samples taken from the ventral sinus via the arthrodial membrane at the base of a walking leg, and on postbranchial haemolymph samples taken from the pericardial sinus surrounding the heart (Butler *et al.* 1978; Taylor & Wheatly, 1979). Samples (0·5 ml) were analysed for oxygen tension (P_{v,O_2} and P_{a,O_2}) using an oxygen electrode enclosed in a thermostated cuvette (Butler & Taylor, 1971), oxygen content (C_{v,O_2} and C_{a,O_2}) using an automatic oxygen analyser (Lexington Instruments), pH (pH_v and pH_a) using a capillary electrode (Radiometer E 5021 a) and total CO_2 (Σ_{v,CO_2} and Σ_{a,CO_2}) using the technique devised by Cameron (1971). From simultaneous measurements of pH and Σ_{CO_2} the CO_2 tension (P_{v,CO_2} and P_{a,CO_2}) values were derived via the Henderson-Hasselbalch equation, as were values for $[HCO_3^-]$, $[CO_3^{2-}]$ and dissolved CO_2 (McMahon, Butler & Taylor, 1978).

Separate prebranchial samples were withdrawn for measurement of lactate concentration using a diagnostic kit as described by Taylor, Butler & Al-Wassia (1977*b*).

Postbranchial haemolymph samples from 6 animals were pooled to give a total of 4–5 ml of haemolymph which was used to construct *in vitro* O_2 equilibrium curves at the pH values measured in water and in air. This was done by equilibrating the haemolymph with various humidified gas mixtures delivered from gas mixing pumps (Wösthoff, Bochum) into an intermittently spinning tonometer (Butler *et al.* 1978).

Variables measured are expressed as mean values ± S.E. with the number of observations in parentheses. Apparent differences between mean values were subjected to Student's *t* test and significance assigned at a confidence level of 95%.

RESULTS

1. *Changes in $\dot{M}_{O_2}$ during 48 h in air*

Mean values of $\dot{M}_{O_2}$ after 1 h and up to 48 h in air (R.H. 80%) at 15 °C for 8 animals of mean mass 41 g are illustrated in Fig. 1. It can be seen that during the first 2 h of aerial exposure there is a progressive rise in $\dot{M}_{O_2}$ to the level established at 2–2½ h which is then maintained over the next 24 h in air, at which point the mean level was 15·6 ± 2·0(8) μmol kg^{-1} min^{-1}. This increase in $\dot{M}_{O_2}$ was consistent but not significant. The limitations of the respirometer only allowed the first reliable measurement of $\dot{M}_{O_2}$ to be taken at 1 h. However, the trend indicates that $\dot{M}_{O_2}$ may have been further limited over the first hour in air. After 48 h in air the $\dot{M}_{O_2}$ was significantly reduced to 11·1 ± 1·3(8) μmol kg^{-1} min^{-1}, which was 60% of its 3 h value in air.

The settled value recorded in air is similar to that interpolated from the regression line of $\dot{M}_{O_2}$ against mass for animals submerged in normoxic water at 15 °C, which a 40 g animal was 13·2 μmol kg^{-1} min^{-1} (Taylor & Wheatly, 1980).

$\dot{M}_{O_2}$ on return to water following 24 h of aerial exposure was measured on further 6 animals of mean mass 62 ± 4 g. There was a significant elevation in $\dot{M}_{O_2}$ for the first 2–2½ h, initially to a level of 23·4 ± 1·9(6) μmol kg^{-1} min^{-1}. After 8 h recovery in water, $\dot{M}_{O_2}$ had fallen to 9·8 ± 0·9(6) μmol kg^{-1} min^{-1}; this is not significantly different from the value of 11·0 μmol kg^{-1} min^{-1} which can be interpolated for a mean mass of 60 g from the line of $\dot{M}_{O_2}$ with mass given in Taylor & Wheatly (1980). When compared with crayfish recovering from 3 h of aerial exposure (Taylor & Wheatly, 1980) the elevation in $\dot{M}_{O_2}$ is of similar magnitude and recovery time is equivalent, indicating that there is no progressive accumulation of an oxygen debt and that the transient increase is likely to result from the disturbance associated with replacement in water.

2. *Heart rate and branchial ventilation*

Heart rate (f_H) was measured on recovery from initial disturbance, followed by entry into air for 24 h and recovery in water, in 6 animals of mean mass 42 ± 4 g (range 25–54 g). Following recovery from initial disturbance, f_H settled to a mean rate of 85 ± 3(6) beats min^{-1} after 2 h in air, which is similar to the rate established in animals submerged in normoxic water at 15 °C (84 ± 3(9) beats min^{-1}). During prolonged exposure in air, f_H progressively decreased with time so that the mean value at 24 h was 71 ± 5(6) beats min^{-1} (see Fig. 1). Animals which had experienced 48 h of aerial exposure exhibited a steady f_H at a mean level of 69 ± 3(4) beats min^{-1}; the reduction below the submerged level was by this time significant (P = 0·02–0·01). Intermittent beating and arrhythmia were observed immediately prior to death, after periods of more than 72 h aerial exposure. On return to water following 24 h of aerial exposure, f_H was initially elevated to 115 ± 7(6) beats min^{-1}, which was 1·4 times the settled submerged value. This was of a similar magnitude to the increase on entry into air and may thus represent a response to the disturbance experienced by the animal during transfer into a new respiratory medium. For up to 3 h following resubmersion, f_H remained significantly elevated above the submerged level, after which time recovery was complete (Fig. 1).

Cardiac output ($\dot{V}_b$) derived from measurements of $\dot{M}_{O_2}$, C_{a,O_2} and C_{v,O_2} according to the Fick principle, was maintained close to the submerged level of 83 ml kg^{-1} min^{-1} during aerial exposure. After 24 h in air, $\dot{V}_b$ was 92 ml kg^{-1} min^{-1} and, as a consequence of the reduction in f_H at this time, calculated stroke volume increased from the submerged level of 0·98 ml up to 1·30 ml. The further reduction in f_H at 48 h was accompanied by a reduction in $\dot{V}_b$ to 78 ml kg^{-1} min^{-1} with stroke volume at 1·13 ml. The tachycardia on initial recovery in water, following 24 h in air, was associated with an increase in $\dot{V}_b$ to 108·5 ml kg^{-1} min^{-1} with stroke volume at 0·94 ml. After 8 h in air, $\dot{V}_b$ had fallen to 64·3 ml kg^{-1} min^{-1} and stroke volume to 0·75 ml.

Respiratory frequency (f_R) was measured from 6 animals of mean mass 30 ± 2 g (range 26–36 g). On entry into air, there was an increase in f_R to 116 ± 13(6) beats min^{-1}, which was 1·75 times the settled submerged value, and it remained significantly elevated for 3 h, then resumed a continuous steady rate of 69 ± 6(6) beats min^{-1}, similar to the settled submerged value of 66 ± 6(6) beats min^{-1}. On return to water, there was once more a transient increase in f_R to 95 ± 12(5) beats min^{-1}, which returned to a settled level within 1 h (Fig. 1). Animals that had been kept in air for 4

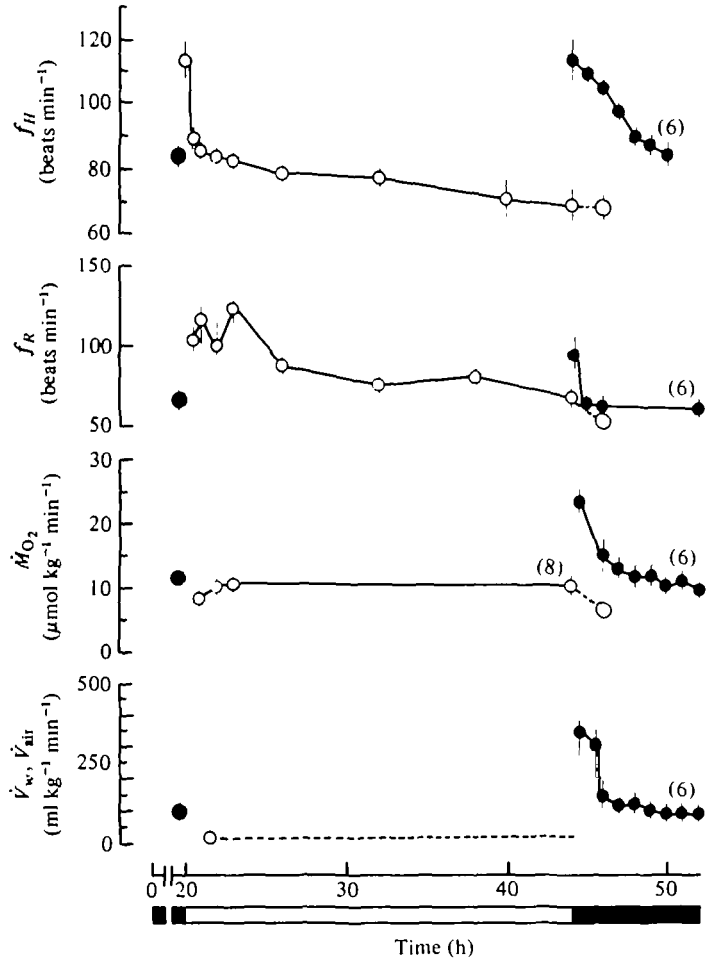

Fig. 1. Mean changes in heart rate (f_H), frequency of scaphognathite movements (f_R), oxygen uptake ($\dot{M}_{O_2}$) and ventilation volume in water ($\dot{V}_w$) or air ($\dot{V}_{air}$) at 15 °C in crayfish allowed to settle in normoxic water for 18 h, followed by 24 h exposure in air then resubmersion in normoxic water, with the changes followed for a further 8 h. The bar below the time-base indicates time in water as a shaded area and time in air as an unshaded area. The initial filled symbol denotes the settled value after 18 h in normoxic water, the open symbols joined by continuous lines are the values in air over a 24 h period, the large open symbol joined by a divided line is the mean value after 48 h in air and the filled symbols joined by continuous lines are the values on recovery in normoxic water for 8 h following 24 h exposure in air. The vertical lines through each value are ± 1 S.E. of mean, where no line is given the S.E. is within the point. The number of animals observed is given in parentheses at the end of each line. As $\dot{V}_{air}$ was only measured at 3 h, this value is indicated by a broken line for the period of aerial exposure.

showed a slight reduction in f_R to 54 beats min^{-1}, and ventilation became intermittent immediately prior to death, after more than 72 h in air.

The rate of branchial ventilation in air ($\dot{V}_{air}$) may be estimated from f_R using the values for $\dot{V}_{air}$ that were measured directly after 3 h aerial exposure by Taylor & Wheatly (1980), assuming that respiratory stroke volume remains constant when the animal is in air. On entry into air, branchial ventilation fell to 7% of its value in ttled submerged crayfish. Over a period of 24 h of aerial exposure this was further

reduced to 4%. Animals exposed to air for 48 h may exhibit even greater reduction in $\dot{V}_{air}$ by virtue of a decrease in f_H. These low values for $\dot{V}_{air}$ are consistent with the availability of O_2 in air as discussed by Taylor & Wheatly (1980).

On return to water after 24 h of aerial exposure, there was a significant hyperventilation with $\dot{V}_w$ (calculated via the Fick principle as described by Butler *et al.* 1978) increasing to $342 \pm 67(6)$ ml kg^{-1} min^{-1}, which was 3·5 times the settled submerged value. The rate remained significantly elevated for 1·5 h and thereafter settled to a level of $80 \pm 18(6)$ ml kg^{-1} min^{-1}, which was not significantly different from the submerged level of $98 \pm 10(17)$ ml kg^{-1} min^{-1} (Fig. 1). It was noted that $\dot{V}_w$ showed great variability between animals when they were first returned to water. Although $\dot{V}_w$ increased to a higher level during recovery from 24 h in air than following 3 h in air (Taylor & Wheatly, 1980), recovery was just as rapid.

3. *Analysis of haemolymph samples*

Blood gas analysis was performed on pre- and postbranchial haemolymph samples from separate groups of 8 animals of comparable mass (overall mean 31 ± 1 g) 1·75, 3, 13·5, 24·5 and 49 h after exposure to air at 15 °C, then at 0·75 and 8 h after return to normoxic water, following 24 h of aerial exposure. The measured values are compared with the values from animals settled in normoxic water at 15 °C provided by Taylor & Wheatly (1980).

(*a*) *Acid–base balance and haemolymph lactate concentration*

The effect of up to 48 h aerial exposure and subsequent resubmersion on pH, P_{CO_2} and the various forms in which dissolved CO_2 exists in solution are illustrated in Fig. 2.

Mean P_{a,CO_2} and P_{v,CO_2} both increased abruptly from $3{\cdot}03 \pm 0{\cdot}24(9)$ and $3{\cdot}53 \pm 0{\cdot}28$ (9) mmHg to $8{\cdot}46 \pm 0{\cdot}48(7)$ and $8{\cdot}39 \pm 0{\cdot}42$ (8) during the initial period of aerial exposure when they were 2·8 and 2·4 times their submerged values respectively, both of these increases being significant. The CO_2 tensions continued to rise over the next 12 h, by which time they had reached levels of $10{\cdot}62 \pm 1{\cdot}08(6)$ and $10{\cdot}98 \pm 1{\cdot}93(6)$ mmHg, which were 3·5 and 3·1 times the submerged values. After 24 h in air, CO_2 tension had dropped slightly to levels of $7{\cdot}84 \pm 0{\cdot}87(6)$ mmHg and $7{\cdot}47 \pm 0{\cdot}86(8)$ mmHg, which were 2·5 and 2·1 times the initial values and still significantly elevated.

Within the first 1–2 h of aerial exposure, both pH_a and pH_v showed a markedly significant acidosis, decreasing from mean values of $7{\cdot}896 \pm 0{\cdot}024(9)$ and $7{\cdot}861 \pm 0{\cdot}011(9)$ respectively in settled submerged crayfish to values of $7{\cdot}457 \pm 0{\cdot}038(7)$ and $7{\cdot}455 \pm 0{\cdot}034(8)$. However, over the ensuing 24 h, the pH of the haemolymph rose progressively so that by 24 h the values recorded were $7{\cdot}786 \pm 0{\cdot}016(6)$ and $7{\cdot}799 \pm 0{\cdot}021(8)$. In the case of the prebranchial haemolymph, this represented a complete recovery to pre-emerged levels, whereas in the arterial haemolymph the value was still significantly below the settled submerged level, although much less acidotic than during the initial period of aerial exposure.

During the first 1–2 h of aerial exposure $[HCO_3^- + CO_3^{2-}]$ concentrations in the pre- and postbranchial haemolymph did not change significantly from the settled submerged levels of $6{\cdot}91 \pm 0{\cdot}42(9)$ and $7{\cdot}49 \pm 0{\cdot}54(9)$ m-equiv l^{-1} respectively. After 12 h in air, the levels had, however, risen to $12{\cdot}59 \pm 0{\cdot}36$ and $12{\cdot}68 \pm 0{\cdot}42(4)$ m-equ

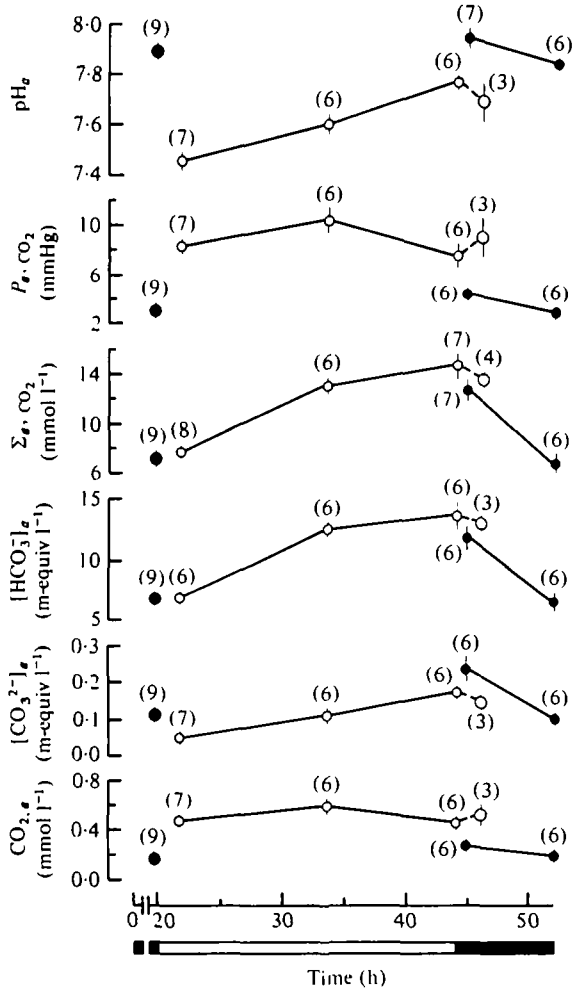

Fig. 2. Changes in the mean values (±S.E. of mean) of the factors governing acid–base status in the postbranchial haemolymph of the crayfish during 24 h exposure in air and subsequent recovery in normoxic water. The traces are from above downwards: pH_a, pH of the haemolymph; P_aCO_2, carbon dioxide partial pressure; Σ_aCO_2, total CO_2 measured by acidification of the haemolymph; $[HCO_3^-]_a$, concentration of bicarbonate ions; $[CO_3^{2-}]_a$, concentration of carbonate ions; $CO_{2,a}$, dissolved CO_2. The bar beneath the time-base and the various symbols are as described in the caption to Fig. 1, with the number of animals observed given in parentheses above each point.

l^{-1}, which were 1·8 and 1·7 times their initial values and were significant increases. By 24 h in air, the $[HCO_3^- + CO_3^{2-}]$ levels were further elevated, most noticeably in the prebranchial haemolymph, where the level was 15·36 ± 0·99(7) m-equiv l^{-1}, which was 2·1 times the settled submerged level.

There was a reversal in all these trends after a period of 48 h aerial exposure. Both arterial and venous haemolymph exhibited a secondary acidosis. There were increases in CO_2 tension and slight reductions in the $[HCO_3 + /[CO_3^{2-}]$ concentrations (Fig. 2). None of these was a significant change with respect to the situation established after 24 h aerial exposure.

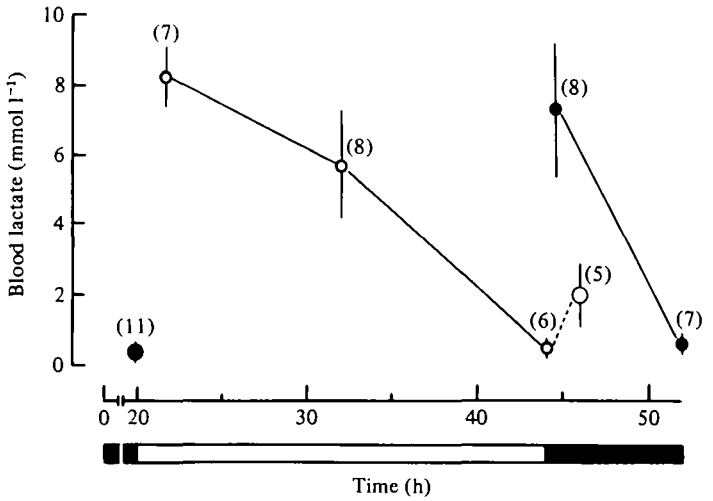

Fig. 3. Mean changes ± S.E. in lactate concentration in the haemolymph of crayfish submerged in normoxic water (●) and during exposure in air (○). The bar beneath the time-base and the various symbols are as described in the caption to Fig. 1.

The changes in acid–base status associated with aerial exposure in the crayfish are not solely attributable to a respiratory acidosis resulting from accumulation of CO_2 when the gills are removed from water. The animals also accumulated lactate ions in the haemolymph when first exposed in air (Fig. 3) with the concentration rising from $0{\cdot}55 \pm 0{\cdot}15(11)$ mmol l^{-1} in submerged animals up to $8{\cdot}28 \pm 0{\cdot}86(7)$ mmol l^{-1} after 1–2 h in air, an increase of 15 times. The associated hydrogen ions may cause a metabolic acidosis in the haemolymph which will summate with the respiratory acidosis to cause the sharp reduction in pH observed on initial exposure in air (Fig. 2). The lactate levels in the haemolymph subsequently decreased, whilst the animals remained in air, to $0{\cdot}57 \pm 0{\cdot}16(6)$ mmol l^{-1} after 24 h, and this decrease may in turn contribute to the apparent compensation for the initial acidosis in air (Fig. 2).

The relative contributions of the respiratory and metabolic sources of the acidosis in air may be determined with the aid of a diagram relating haemolymph pH to $[HCO_3^- + CO_3^{2-}]$ and including isopleths for P_{CO_2} as described by Davenport (1969). Diagrams for pre- and postbranchial haemolymph illustrating the progressive changes in acid–base status with time before, during, and after 24 h aerial exposure at 15 °C are given in Fig. 4. These diagrams also contain a buffer line obtained by equilibrating haemolymph with gas mixtures of known P_{CO_2} *in vitro*. The slope of this line plots the pH change associated with accumulation of CO_2 and is recognized as a measure of the amount of H^+ bound by non-bicarbonate buffers (Piiper, Meyer & Drees, 1972; Truchot, 1978). Deviations from this line indicate the probable contribution of H^+ associated with lactate ions, i.e. the metabolic acidosis. By employing the analysis of these diagrams described by Davenport (1969) and more recently by Wood, McMahon & McDonald (1977) it is possible to discriminate the relative contributions of CO_2 and lactate to the measured changes in pH. After 1·75 h in air the marked acidosis, with postbranchial pH changing from $7{\cdot}896 \pm 0{\cdot}024(9)$ in settled submerged animals down to $7{\cdot}457 \pm 0{\cdot}038(7)$, was predominantly due to H^+ ions associated with the accumulation of lactate ions which accounted for 70% of the change, only 30%

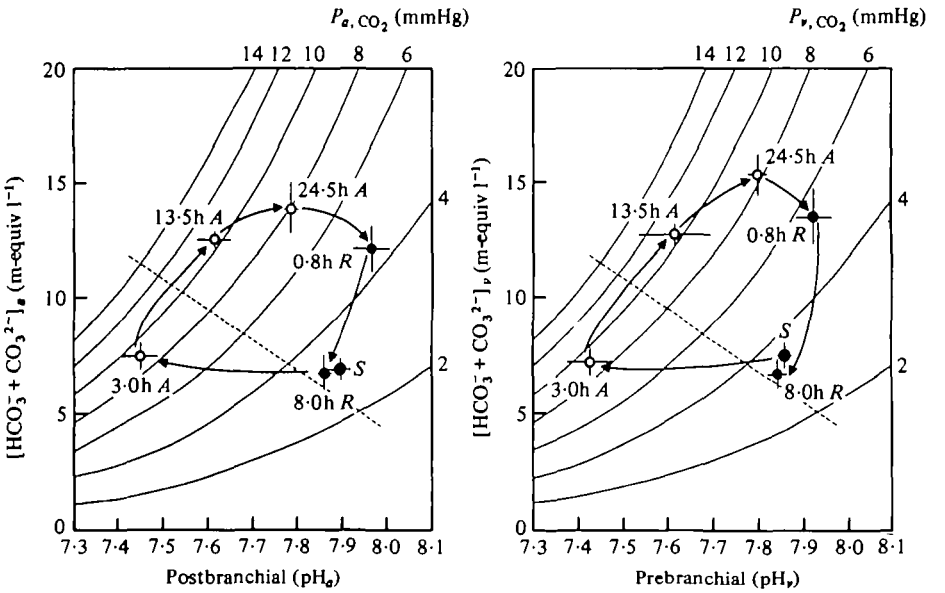

Fig. 4. Variation in mean pH and $[HCO_3^- + CO_3^{2-}]$ levels (±S.E. of mean) in the postbranchial and prebranchial haemolymph of the crayfish settled in normoxic water (*S*), during 24 h exposure in air (*A*) and subsequent recovery on resubmersion (*R*) at 15 °C. The time periods from emersion and resubmersion are given beside each point. The thin continuous lines are isopleths for P_{CO_2} levels over the range of variation observed. The oblique broken line traces the buffer line calculated from two *in vitro* pH and $[HCO_3^-]$ measurements at different P_{CO_2} levels.

being attributable to CO_2. Similarly, the compensation for the initial acidosis, with postbranchial pH recovering to 7·786 ± 0·016(6) after 24 h in air, was due to the marked reduction in lactate levels, which again accounted for 70% of the change. The other 30% was due to accumulation of HCO_3^- ions at constant P_{CO_2}, with a resultant increase in the bicarbonate buffering capacity of the haemolymph, which served to compensate for the respiratory acidosis (Truchot, 1975).

Those animals which were left for a further 24 h in air exhibited a secondary increase in circulating lactate to 2·03 ± 0·98(5) mmol l^{-1}, which was 4 times the settled submerged value. This was a significant elevation but not of the same magnitude as that recorded on entry into air.

After 24 h of aerial exposure, the crayfish were returned to normoxic water at 15 °C and the same variables measured. The recovery to a settled submerged acid–base status was rapid, being complete within 8 h. Recovery was accompanied by an initial alkalosis in both the post- and prebranchial haemolymph, where pH values of 7·963 ± 0·042(7) and 7·924 ± 0·042(6) were recorded following 45 min recovery in aerated water. This increase was not significant. Both P_{a,CO_2} and P_{v,CO_2} were reduced to levels of 4·60 ± 0·48(6) and 5·43 ± 0·41(6) mmHg, which were not significantly above those measured in submerged animals within the first hour of resubmersion, and decreased slightly over the ensuing 8 h to 3·13 ± 0·33(6) and 3·27 ± 0·25(7) mmHg. The $[HCO_3^- + CO_3^{2-}]$ values recorded in the first hour of recovery were still high at 12·16 ± 1·02(6) and 13·47 ± 1·25(6) m-equiv l^{-1}, which were approximately 1·8 times submerged level in both post- and prebranchial haemolymph. These levels were

not significantly elevated, however, and were restored to levels of $6{\cdot}69 \pm 0{\cdot}78(6)$ and $6{\cdot}74 \pm 0{\cdot}58(6)$ m-equiv l^{-1} within 8 h recovery, which were comparable with submerged values.

On resubmersion in water following 24 h of aerial exposure, the lactate concentration recorded within the first hour was $7{\cdot}29 \pm 1{\cdot}86(8)$ mmol l^{-1} which was 13 times the settled submerged value. The appearance of high concentrations of lactate ions in the haemolymph during the first stages of recovery from 24 h aerial exposure would be expected to shift the pH value at 24 h (7·786) to 7·615 due to a metabolic acidosis. At this point there is instead an alkalosis (Fig. 2), with measured pH at $7{\cdot}963 \pm 0{\cdot}042$ (7): this is possibly caused by a 'wash-out' of CO_2 consequent upon the period of hyperventilation on resubmersion (Fig. 2), whilst HCO_3^- concentration remains high (Figs. 2, 4).

During further recovery in normoxic water, lactate levels fell to $0{\cdot}68 \pm 0{\cdot}23(7)$ mmol l^{-1} within 6 h, which was similar to that measured on settled submerged animals (Fig. 3). The reduction in the initial alkalosis on resubmersion can be attributed to the marked reduction in $[HCO_3^- + CO_3^{2-}]$ which would contribute a reduction of 0·265 pH units, which was contested by a potential increase of 0·15 pH units arising from the reduction in the concentration of lactate ions.

(b) Haemolymph oxygen tension and content

Immediately upon exposure to air the values for pre- and postbranchial oxygen tension, P_{v,O_2} and P_{a,O_2}, were significantly reduced to 49% and 32% of their settled submerged values. This internal hypoxia continued throughout the period in air and after 24 h mean P_{v,O_2} was $9 \pm 1{\cdot}5(3)$ mmHg and P_{a,O_2} was $10 \pm 0{\cdot}5(5)$ mmHg (Fig. 5).

The values for pre- and postbranchial oxygen content, C_{v,O_2} and C_{a,O_2}, dropped virtually to zero when the animals were first taken into air, so that $a-v_{O_2}$ content difference fell to $0{\cdot}02 \pm 0{\cdot}01$ mmol l^{-1} after 1·75 h in air. There followed a progressive recovery in both values so that after 24 h C_{v,O_2} was $0{\cdot}12 \pm 0{\cdot}02(8)$ and C_{a,O_2} was $0{\cdot}28 \pm 0{\cdot}06(8)$ mmol l^{-1}, levels not significantly lower than those measured on submerged animals, and $a-v_{O_2}$ content difference was restored to $0{\cdot}16 \pm 0{\cdot}04$ mmol l^{-1} (Fig. 5). After 48 h in air, there was a secondary decrease in O_2 content which was not, however, significant.

This apparent discrepancy between the changes in oxygen tension and content in the haemolymph during aerial exposure may be explained by the Bohr shift, which occurs in the haemolymph due to the initial acidosis on exposure to air, as described above. The oxygen equilibrium curves for the haemolymph taken from crayfish submerged in normoxic water (mean pH_a of 7·855) and following 3 h in air (mean pH_a of 7·400) are illustrated in Fig. 6. The P_{50} rose from 8 mmHg to 14·5 mmHg after 3 h in air.

Within the initial period of aerial exposure when the haemolymph was relatively acid, the low mean P_{a,O_2} value ($10{\cdot}5 \pm 1{\cdot}2$ mmHg) was well below the P_{50} value of haemocyanin (being equivalent to a value of 30% from Fig. 6). Following the progressive compensation for this acidosis the partial removal of the Bohr shift restored the low P_{a,O_2} after 24 h in air ($10{\cdot}1 \pm 0{\cdot}5$ mmHg) to a level above the P_{50} value of the haemocyanin (approximately 70% from Fig. 6). This had the effect of progressiv

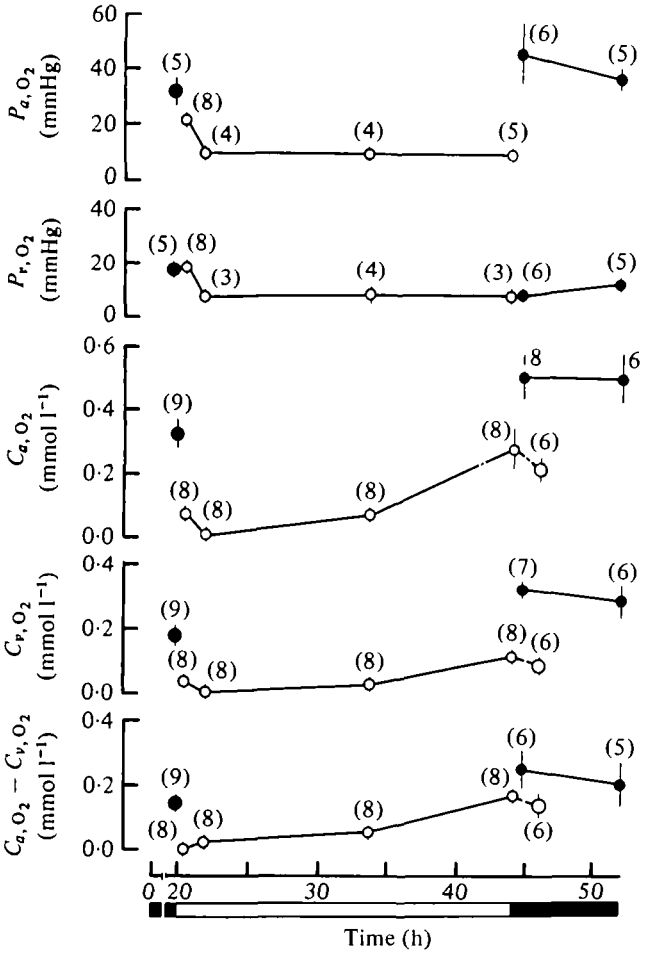

Fig. 5. Changes in the mean values (±S.E. of mean) of oxygen tension ($P_{a.O_2}$ and $P_{v\ O_2}$), oxygen content ($C_{a.O_2}$ and $C_{v.O_2}$) and the oxygen content difference ($C_{a.O_2}$–$C_{v.O_2}$) in the post-branchial and prebranchial haemolymph of the crayfish submerged in normoxic water (●) and exposed in air (○) at 15 °C. The bar beneath the time-base and the various symbols are as described in the caption to Fig. 1. The number of animals observed is given in parentheses above each point.

restoring the C_{a,O_2} value towards its submerged value and partially replenishing the venous store of oxygen present in the prebranchial haemolymph.

Following replacement in water after 24 h in air, P_{a,O_2} increased to 47·7 ± 11·6(6) mmHg, which was 44% higher than the settled submerged value, though this increase was not significant. P_{v,O_2} initially decreased to 8·6 ± 1·1(6) mmHg, which represented 48% of the settled submerged value; this was a significant reduction. After a period of 8 h the values for P_{a,O_2} and P_{v,O_2} had returned to levels of 38·4 ± 3·2(5) and 12·9 ± 0·7(5) mmHg, which were similar to the submerged values.

Replacement in water following 24 h in air resulted in an increase in C_{a,O_2} to 0·507 ± 0·065(8) mmol l^{-1}, which was 56% above the settled submerged value: this increase was significant. C_{v,O_2} also rose initially to 0·331 ± 0·022 mmol l^{-1}, which was an 82% increase, but this did not prove to be significant. The net result was a

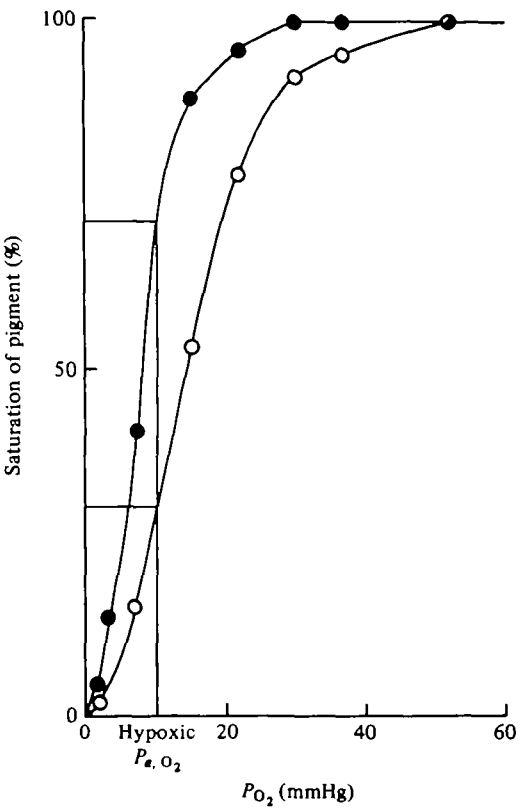

Fig. 6. Oxygen equilibrium curves measured *in vitro* on haemolymph withdrawn from crayfish settled in normoxic water (●) and after 3 h in air (○). The pH was set close to the mean value for each group of animals and was 7·86 and 7·40 respectively. The vertical line at a P_{O_2} of 10 mmHg traces the approximate hypoxic P_{a,O_2} value measured in the haemolymph of crayfish throughout the period of exposure in air and indicates the effect of the Bohr shift on the percentage saturation of the pigment at this P_{O_2}.

slight, though insignificant, increase in $C_{a,O_2} - C_{v,O_2}$ to 0·249 ± 0·052(6) mmol l^{-1}. After 8 h recovery in water the $C_{a,O_2} - C_{v,O_2}$ returned to 0·198 ± 0·073(5) mmol l^{-1}, which approached its initial submerged level prior to aerial exposure, although C_{a,O_2} remained slightly above the settled level at 0·507 ± 0·069 mmol l^{-1}.

DISCUSSION

Crayfish were disturbed by transfer from normoxic water into air (R.H. 80%) at 15 °C and both f_H and f_R were elevated during the first 1–2 h, after which they recovered to the submerged rates (Taylor & Wheatly, 1980). $\dot{M}_{O_2}$ appeared to be depressed at first and then to recover to the submerged rate. This initial depression of $\dot{M}_{O_2}$ was confirmed by measurement of extremely low levels of C_{a,O_2} and C_{v,O_2} from crayfish when first in air.

It is during the initial 1 h period of exposure to air, when the ability to transport oxygen to the tissues failed, that the concentration of lactate ions in the haemolymph rose steeply. Based on the assumption that 1 mole of lactic acid is equivalent to

production of 1·5 mole of ATP from each glycosyl unit of glycogen, then a 50 g crayfish produced approximately 120 μmol of ATP due to accumulation of lactic acid, when first transferred into air. This compares with the loss of approximately 200 μmol of ATP by a 50 g animal within the first 1 h of aerial exposure when $\dot{M}_{O_2}$ is close to zero, on the basis that 6·33 moles of ATP arise from the complete oxidation of glycosyl residues by 1 mole of oxygen. It seems possible, therefore, that the measured level of lactic acid production could fuel the initial disturbance on transfer to air in the absence of any aerobic metabolism. When crayfish were exposed to hypoxic water they accumulated lactate in proportion to the measured reduction in $\dot{M}_{O_2}$ sufficient to partially compensate for the reduction in aerobic ATP production (Wheatly & Taylor, 1981).

As the accumulation of lactate is most likely to follow on from the failure to transport oxygen when first in air, then a factor other than the Bohr shift on the haemocyanin is likely to have caused the initial almost complete failure to transport oxygen, when first exposed in air. The water contained in the branchial chambers when a crayfish is taken into air may take up to 1 h to drain away. This may preclude aerial gas exchange. When the chambers drain, the filaments of the phyllobranchiate gills, recently described by Burggren *et al.* (1974), appear to remain clumped at first, possibly due to the surface tension of the covering film of water. Effective gas exchange with air may be delayed until the gills dry out, although some exchange may occur over the inner wall of the branchiostegite which is well vascularized and thin-walled (J.-C. Massabuau, personal communication), as described in the arid-zone crab *Holthuisana transversa* by Taylor & Greenaway (1979).

When the gills of a water breather are taken into air the animal begins to accumulate Co_2. A sustained increase in P_{CO_2} is observed in the crayfish that is up to 3 times the submerged level. Both the P_{CO_2} and Σ_{CO_2} levels reached in the crayfish when in air resemble the levels measured in the terrestrial crab *Birgus latro* (Cameron & Mecklenberg, 1973) and the terrestrial hermit crab *Coenobita clypeatus* (McMahon & Burggren, 1979). The haemolymph pH values are, however, different in the three species. *Birgus* has a mean pH of 7·54 associated with a calculated $[HCO_3^-]$ of 5·7 m-equiv l^{-1} whilst *Coenobita* has a pH_a of 7·84 and an $[HCO_3^-]$ of 11·0 m-equiv l^{-1}. The mean pH_a 1–3 h after transfer into air in the crayfish was reduced to 7·457 and $[HCO_3^-]$ was 7·2 m-equiv l^{-1}, which was similar to the submerged level. After 24 h in air pH has risen to 7·786 and $[HCO_3^-]$ was 13·7 m-equiv l^{-1}. These figures confirm the link between pH (or H^+ concentration) and $[HCO_3^-]$ described in water breathers such as the shore crab over a range of temperatures (Truchot, 1978). Compensation for the initial respiratory acidosis on exposure to air may, therefore, be accomplished by the elevation of buffer base at constant P_{CO_2}, as was described for the shore crab by Truchot (1975). This parallels the reported increase in the CO_2-combining power of the blood, correlated with an increase in the average level of arterial P_{CO_2}, in a range of fish species showing an increasing reliance on air breathing (Johansen, 1970). As the gills are not in contact with water when the crayfish is taken into air, ion exchange can play no part in the provision of basic ions, which must originate from an internal source of fixed base such as the $CaCO_3$ available in the calcified exoskeleton (Truchot, 1975).

Detailed comparison of the data from the present study with results of the similar

study on *Carcinus* (Truchot, 1975), both studies having been performed at 15 °C, helpful in elucidating the basis for the observed pH changes. Truchot (1975) observed that after 9 h in air the pH of the haemolymph had fallen to 0·23 pH units below the level in submerged crabs. After 24 h in air compensation had reduced this difference to 0·11 pH unit below the submerged level. In the crayfish, the maximum degree of acidosis appeared more rapidly, within 1–2 h after transfer into air, and was 0·44 pH unit below the submerged level. Following compensation, this was reduced to 0·11 pH unit, i.e. identical to the shore crab. A very large component of the initial acidosis in the crayfish is the H^+ ions associated with the increased levels of lactate in the haemolymph. The time course for appearance and subsequent reduction in the high levels of circulating lactate matches the appearance and apparent progressive compensation for the marked acidosis in air.

Carcinus did not accumulate lactate after 3 h in air and it is possible that the rigidity of its gill lamellae is sufficient to resist collapse and maintain aerobic metabolism throughout a period of aerial exposure (Taylor & Butler, 1978). This possibility is supported by the greater ability of *Carcinus* to transfer oxygen from air. The calculated value of T_{O_2} for *Carcinus* after 3 h in air at 15 °C was 4 times higher than for the crayfish (Taylor & Wheatly, 1980).

The progressive reduction in lactate concentration whilst the animals remained in air may be explained by progressive reoxidation to pyruvate. A similar pattern of lactic accumulation and subsequent reoxidation, possibly due to biochemical adjustment, was observed in the lungless salamander *Desmognathus fuscus* exposed to severe hypoxia (Gatz & Piiper, 1979).

Alternatively the lactic acid may leave the haemolymph, to accumulate in the tissues and to be subsequently washed out on initial recovery in water. This could explain the observed appearance of high levels of lactic acid in the haemolymph immediately after resubmersion. Similar increases in lactic acid level on resubmersion following a period in air have been described in the eel (Berg & Steen, 1965) and in the cod by Leivestad, Anderson & Scholander (1957). This response resembles that shown by diving vertebrates such as the seal, which show a bradycardia during laboratory dives and redistribute blood preferentially towards the heart, lungs and C.N.S., with lactic acid accumulating in the muscles to be washed out at the end of the dive (Scholander, Irving & Grimmell, 1942). Recent observations have indicated the possibility of delayed appearance of lactic acid in the haemolymph of land crabs following periods of induced activity (D. J. Randall, personal communication).

The haemolymph of the crayfish shows a marked Bohr shift with changes in acidity having a $\Delta P_{50}/\Delta$pH value of 14·6 compared with 30 for *Cancer magister* (quoted by McMahon & Burggren, 1979) which is a sublittoral crab, 12 for the air breathing land crab *Gecarcinus* (Redmond, 1968) and 16 for *Coenobita* (McMahon & Burggren, 1979). The Bohr shift shown by crayfish haemolymph, although relatively large, more closely resembles that of the air-breathing rather than the fully aquatic species and may represent a pre-adaptation to confer some resistance to the initial pH changes associated with moving into air.

After 24 h in air the crayfish appears to have compensated for the immedate effects of transfer from water and to be maintaining aerobic metabolism satisfactorily without recourse to the venous reserve utilized after 3 h in air (Taylor & Wheatley, 1980).

ociated with the recovery in the oxygen content of the haemolymph, the relative effectiveness of transfer of O_2 into the haemolymph, E_b, increased from 20 after 3 h exposure (Taylor & Wheatly, 1980) up to 34 after 24 h, and T_{O_2} increased from 0·09 to 0·13, which is the same value as that recorded in submerged animals by Taylor & Wheatly (1980). Despite this apparent recovery, survival in air with a relative humidity around 70% at 15 °C is limited to about 72 h; after 48 h there were some noticeable reversals in the patterns of compensation and recovery of the measured variables observed over the first 24 h. The reasons for this incipient breakdown after 48 h in air are not apparent from the present data, but it is likely that it results from the ancillary problem facing all air-breathers, particularly those which retain gills, of desiccation by evaporative water loss and loss of the aquatic route for ion exchange. This may be expected ultimately to affect respiratory gas exchange and acid–base balance. This possibility is at present under investigation.

When, after 24 h in air, crayfish were replaced in normoxic water, the changes in the measured variables with time were complex. Both f_H and $\dot{M}_{O_2}$ were initially elevated, probably in response to the disturbance occasioned by transfer, as discussed by Taylor & Wheatly (1980). There was a significant hyperventilation for the first 3 h in water during which percentage extraction of oxygen from water was maintained relatively high at 30%, possibly by virtue of an increase in T_{O_2} to 0·31 compared to 0·13 in settled submerged animals. The period of hyperventilation led to increased oxygen levels in the haemolymph. Recovery of acid–base status was complicated by the appearance of high concentrations of lactate in the haemolymph on resubmersion. The potential acidosis which this represents was, however, overridden by a respiratory alkalosis due to washout of CO_2 during the period of hyperventilation, combined with the short-term retention of high levels of HCO_3^-.

After 8 h resubmersion, all the measured variables had returned towards their settled submerged values. Truchot (1975) attributed the relatively rapid recovery of acid–base status following resubmersion in *Carcinus* to excretion of excess base across the gills. This possibility, plus the associated fluxes of ions and water consequent upon rehydration after exposure in air, are currently under investigation.

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