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THE PHYSIOLOGY OF DEHYDRATION STRESS IN THE LAND CRAB, *CARDISOMA CARNIFEX*: RESPIRATION, IONOREGULATION, ACID–BASE BALANCE AND NITROGENOUS WASTE EXCRETION

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SUMMARY

Air-breathing *Cardisoma carnifex*, collected in Moorea, French Polynesia, were held in fresh water similar in chemical composition to that in their burrows. Under control conditions, which allowed branchial chamber flushing but not ventilation of the medium, crabs demonstrated net Na^+ and Cl^- uptake, and ammonia, urea and base excretion (= acidic equivalent uptake). Throughout 192 h of water deprivation, crabs dehydrated slowly at a rate of $0{\cdot}55\,g\,H_2O\,kg^{-1}\,h^{-1}$, eventually reaching a near lethal 18% loss of total body water. Increases in haemolymph osmolytes were quite variable (0–29%); electrolyte excretion was negligible. $\dot{M}_{O_2}$ and $\dot{M}_{CO_2}$ both decreased by approximately 55%, maintaining an unusually low gas exchange ratio ($R = 0{\cdot}53$), and suggesting general metabolic depression. There was no evidence of internal hypoxia as haemolymph lactate remained at hydrated levels and Pa_{O_2} actually increased. The dominant acid–base response was a progressive metabolic alkalosis accompanied by a partially compensating rise in Pa_{CO_2}. Alkalosis was probably caused by blockage of the normal aquatic excretion of base produced by the metabolism of this herbivore. Other possible causes were eliminated: i.e. alkalaemia due to contraction of the ECFV; entrainment *via* strong ion shifts; $CaCO_3$ mobilization; and ammonia accumulation in the haemolymph. In the absence of water, net ammonia production and excretion both appeared to cease, and alternate end products (urea, uric acid) did not generally accumulate. Within 2 h of rehydration, crabs regained more than half the lost water, $\dot{M}_{O_2}$ and $\dot{M}_{CO_2}$ increased above control levels, and ammonia excretion and haemolymph concentration both exhibited a prolonged (56 h) 4- to 6-fold rise. At the same time, metabolic alkalosis was reversed in association with elevated net base excretion into the water; the latter was correlated with an increase in the strong ion difference (SID) flux ($[Na^+ + K^+ + Ca^{2+} + Mg^{2+} - Cl^-]$).

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INTRODUCTION

Dehydration is thought to be the single most important factor limiting the success of decapod crustaceans on land (Powers & Bliss, 1983). There exist numerous studies on the effects of dehydration on osmotic and ionic balance in terrestrial crabs (for reviews, see Bliss, 1968, 1979; Mantel, 1979; Mantel & Farmer, 1983) but its consequences for respiration, blood gas transport and acid–base balance have received scant attention. Notable exceptions are studies by Gross (1955) on *Pachygrapsus*, MacMillen & Greenaway (1978) on *Holthuisana* and Burggren & McMahon (1981) and Wheatly, Burggren & McMahon (1984) on *Coenobita*, *Birgus* and *Cardisoma*. The goal of the present study was to extend these observations with particular reference to interactions between gas exchange, electrolyte balance, acid–base status and nitrogen metabolism during dehydration stress, for these processes are all inter-related (Stewart, 1978; Atkinson & Camien, 1982; Hochachka & Somero, 1984).

The terrestrial gecarcinid *Cardisoma carnifex* was chosen for study because its basic physiology has been well characterized as a result of the *Alpha Helix* expedition to the Palau Islands (see Cameron, 1981*a* for overview). Aerial gas-exchange occurs *via* a complex 'lung'/gill apparatus enclosed within large branchial chambers which are ventilated by forward scaphognathite pumping (Wood & Randall, 1981*a*,*b*). The gills are also putative sites of ion, acid–base and nitrogenous waste exchange (Randall & Wood, 1981; Towle, 1981) and are normally bathed in water carried at the base of the chambers during terrestrial excursions. When an external pool is provided, the branchial chamber water is changed every few minutes. This habit makes *C. carnifex* especially amenable to flux studies, for exchange rates of various substances between the whole crab and its environment can be determined by periodic analysis of a closed external pool.

Recently, we have studied a population of *C. carnifex* in the wild in Moorea, French Polynesia (Wood & Boutilier, 1985). Dehydrated, apparently distressed, crabs were occasionally seen above ground during the daytime. However, despite their evolutionary origin in sea water, the crabs usually retreat to burrows containing fresh water during the heat of the day, apparently to avoid such dehydration. The crabs are essentially in osmotic and ionic 'equilibrium' with this fresh water when caught foraging above ground at night. Ammonia is the major nitrogenous waste, and uric acid production appears negligible. The present investigation focused on the following questions.

(1) Does O_2 consumption decline during dehydration (cf. Gross, 1955; MacMillen & Greenaway, 1978)? Is CO_2 excretion similarly affected? Branchial water is probably a significant route of CO_2 excretion in *C. carnifex*, and the overall gas exchange ratio in hydrated animals is curiously low ($R = 0{\cdot}5$–$0{\cdot}6$), perhaps indicating the fixation of respiratory CO_2 as carbonate deposition in the exoskeleton (Wood & Randall, 1981*a*; Randall & Wood, 1981; Cameron & Wood, 1985).

(2) If O_2 consumption is depressed, is there an accompanying internal hypoxia? Lactic acid accumulation could explain the metabolic acidosis observed in desiccated

crabs by Burggren & McMahon (1981). Alternative explanations include a blockage of the acidic equivalent excretion which must occur if respiratory CO_2 is fixed as carbonate in the carapace (Cameron & Wood, 1985), or an entrainment of acidosis *via* strong electrolyte shifts accompanying dehydration, as suggested by Wheatly *et al.* (1984).

(3) In light of the above, how does haemolymph osmotic and ionic status change during dehydration, and is ionic and acidic equivalent exchange with the environment altered during rehydration?

(4) As excretion of gaseous ammonia to the atmosphere apparently does not occur (Wood & Boutilier, 1985), what happens to nitrogenous waste metabolism during long-term deprivation of water? Internal ammonia accumulation would be expected to cause alkalosis and toxicity, while conversion to alternative end products (e.g. urea, uric acid) could correct the problem (Atkinson & Camien, 1982).

MATERIALS AND METHODS

Experimental animals

Land crabs (*Cardisoma carnifex*; 90–330 g) were collected by hand at night within 0·5 km of the shore on the island of Moorea, French Polynesia, during July and August. In the laboratory (temperature = 24–26°C), they were held communally in large plastic tubs containing 1 cm of fresh water (4 l kg^{-1}), which was changed daily. This fresh water, which was identical to that used in the subsequent experiments, was prepared as a 1% sea water: 99% Moorea tap water (non-chlorinated) mixture. Levels of major electrolytes (in mequiv l^{-1}) were $[Na^+] \approx 6{\cdot}0$, $[Cl^-] \approx 6{\cdot}0$, $[Mg^{2+}] \approx 1{\cdot}2$, $[Ca^{2+}] \approx 0{\cdot}5$, $[K^+] \approx 0{\cdot}2$, $[NH_4^+] \approx 0{\cdot}01$, with osmolality $\approx$ 20 mosmol kg^{-1}, titration alkalinity (to pH = 4·0) $\approx$ 1·0 mequiv l^{-1}, and pH $\approx$ 7·0. This composition was very similar to fresh water found in natural crab burrows in Moorea (Wood & Boutilier, 1985). Approximately 48 h prior to the start of experiments (4–8 days after capture), the crabs were towel-dried and weighed (to an accuracy of 0·2 g) and the chelipeds were firmly taped closed. This procedure had no apparent ill effect on the crab and was undoubtedly beneficial to the investigators. The larger crabs (252 ± 14 g; $\bar{X} \pm 1$ S.E.M.; $N = 9$) were assigned to the haemolymph series in which blood parameters were measured, and were fitted with arterial sampling sites covered with dental dam over the anterior margin of the pericardium, as described by Wood & Randall (1981*a*). The smaller crabs (128 ± 9 g; $N = 8$) were assigned to the gas exchange series.

The animals were then transferred to their individual experimental chambers, which were dark polyethylene buckets (22 cm diameter × 25 cm depth) in the haemolymph series, or blackened sealable respirometer jars (12 cm diameter × 14 cm depth) in the gas exchange series. The lids were left slightly ajar and heavily weighted to prevent escape. The chambers were filled with fresh water to a depth of $\approx$1 cm (2 l kg^{-1} in the haemolymph series and 1·2 l kg^{-1} in the gas exchange series

because of the different vessel dimensions). This depth allowed the crabs to draw water into their branchial chambers through the posterior margins of the brachiostegites, as described by Wood & Randall (1981*a*), but not to ventilate it. The water in the buckets and jars was renewed at approximately 12-h intervals.

Experimental protocols

The two experimental series were designed to correlate changes in haemolymph gases, ions, acid–base status, nitrogenous wastes and fluxes with the environmental water (haemolymph series) with changes in O_2 and CO_2 exchange (gas exchange series) under control conditions, during progressive dehydration and during subsequent rehydration. Thus experimental protocols and sampling times were virtually identical in the two series.

Haemolymph series

After 48 h recovery from handling and surgery, a control flux determination was performed: new fresh water was introduced and water samples were taken at 15 min (to allow initial flushing of the branchial chambers) and again after 12 h. Fluxes determined in this way represent exchanges across the total body surface (i.e. gills, gut, renal system, etc.), although, as the crabs had been starved in the laboratory, defaecation did not occur. An arterial haemolymph sample (0·5 ml) was removed with minimal disturbance, the animal was towel-dried, weighed again, and placed in a clean, dry bucket. This point represented time 0, the start of dehydration. The blood sampling and weighing procedures were repeated at 36-h intervals until 180 h. At 192 h, the crab was weighed again without blood sampling, and the bucket was thoroughly flushed with exactly 200 ml of distilled water to recover all electrolytes and wastes lost by the animal during 8 days of dehydration. The crab was then returned to hydrated conditions (time 0, rehydration) with haemolymph samples drawn at 2 and 36 h of rehydration. Weights were determined at 2, 36 and 72 h, and flux determinations were performed in the standard fashion over the periods 0–2, 2–19, 19–36 and 36–53 h of rehydration.

Gas exchange series

These experiments were designed to measure total (i.e. from both air and water) oxygen consumption ($\dot{M}_{O_2}$) and CO_2 excretion ($\dot{M}_{CO_2}$) rates at comparable times to the blood samples in the first series during the dehydration/rehydration protocol. As these determinations took 4–6 h, they actually spanned the haemolymph sample times of the first series, i.e. control, 33–39, 69–75, 105–111, 141–147 and 177–183 h of dehydration, 0–4 and 33–39 h of rehydration. For each measurement, initial air and water samples (if present) were taken, the respirometer was then sealed and final air and water samples were drawn after 4–6 h, through appropriate ports. Weights were determined at 192 h dehydration (by weighing the dry jar and crab) and after

rehydration (by weighing the towel-dried crab) for comparison with the haemolymph series.

Analytical techniques

Haemolymph samples were drawn anaerobically into ice-cold, gas-tight Hamilton syringes and analysed immediately for pHa, Pa_{CO_2}, Pa_{O_2} and Ca_{CO_2} using standard Radiometer electrode methodology (Cameron, 1971; Boutilier, Randall, Shelton & Toews, 1978; Wood & Randall, 1981*a*). Wösthoff pumps provided precision gas mixtures for calibration. Coulometric titration with a Radiometer CMT 10 chloridometer was used to determine haemolymph $[Cl^-]$, and a 100-μl sample was fixed in 200 μl ice-cold 8% perchloric acid for subsequent measurement of lactate. The remainder of the haemolymph sample (including that recovered from the electrode chambers) was frozen at −20 °C for transport home, and subsequent determination of $[Na^+]$, $[K^+]$, $[Mg^{2+}]$, $[Ca^{2+}]$, osmolality, total protein, total ammonia, urea, uric acid and glucose. In our experience, freezing does not appreciably affect these parameters in crab blood. Clots were removed by centrifugation prior to analysis.

Air and water samples from the gas exchange series were analysed for P_{O_2} and P_{CO_2} using the same Radiometer electrode system. The total CO_2 content of water samples was determined by a modification of the Cameron (1971) technique in which stronger acid (0·1 mol l^{-1} HCl), a larger sample (200 μl) and a more dilute standard (3 mmol l^{-1} $NaHCO_3$) were employed to enhance sensitivity. Water from the flux experiments was analysed immediately for titratable alkalinity by titration of air-equilibrated samples to pH = 4·00 with 0·02 mol l^{-1} HCl as described by McDonald & Wood (1981). The remainder of the sample was frozen for later determination of $[Na^+]$, $[Cl^-]$, $[K^+]$, $[Mg^{2+}]$, $[Ca^{2+}]$, total ammonia, urea and uric acid.

$[Na^+]$ and $[K^+]$ were measured by flame photometry (Eel Mk II or Radiometer FLM3), osmolality was measured by vapour pressure osmometry (Wescor 5100B), $[Cl^-]$ by coulometric titration (Radiometer CMT 10 or Buchler Cotlove 4-2000, the latter for a few very dilute water samples), $[Mg^{2+}]$ by atomic absorption spectrophotometry (Varian 1275AA) and $[Ca^{2+}]$ either by atomic absorption (water samples) or by colourimetric reaction with *o*-cresolphthalein complexone using Sigma (1981*a*) reagents (haemolymph samples). The two $[Ca^{2+}]$ techniques were cross-validated. Micro-modifications of commercial diagnostic kits were used for the spectrophotometric assay of lactate (lactic dehydrogenase/NADH method; Sigma, 1977), glucose (*o*-toluidine method; Sigma, 1980), urea (diacetylmonoxine method; Sigma, 1981*b*), uric acid (uricase/phosphotungstate method; Sigma, 1981*c*), haemolymph total protein (Lowry method, Sigma, 1982*a*) and haemolymph total ammonia (L-glutamic dehydrogenase/NAD method; Sigma, 1982*b*). Total ammonia in water was determined by a micro-modification of the salicylate hypochlorite method of Verdouw, van Echteld & Dekkers (1978). Different ammonia assays were used for water and haemolymph as the simpler salicylate hypochlorite method gave spurious values for haemolymph; the two assays were cross-validated on water samples.

Calculations

Haemolymph $[HCO_3^-]$ was calculated as $Ca_{CO_2}-(\alpha CO_2 \times Pa_{CO_2})$ using αCO_2 at the appropriate temperature and haemolymph ionic strength from Truchot (1976). Operational pK′ values (combining HCO_3^- and CO_3^{2-}) for construction of $[HCO_3^-]_a$ *vs* pHa diagrams (e.g. Fig. 5) were calculated using the Henderson–Hasselbalch equation. The change in the concentration of acidic/basic equivalents in the haemolymph was calculated as:

$$\Delta H_h^+ = [HCO_3^-]_i - [HCO_3^-]_f + \beta(pHi-pHf), \quad (1)$$

where i and f refer to initial and final values and β is the slope of the haemolymph non-bicarbonate buffer line, taken as 12·1 slykes for *C. carnifex* from Wood & Randall (1981*b*). β is a function of total protein (mainly haemocyanin) concentration, and as this exhibited only small changes during the present experiments (see Fig. 7), the error introduced by assuming a constant value of β is negligible. O_2 saturation of the haemolymph was estimated from measured values of Pa_{O_2} and pHa and a family of haemocyanin O_2 dissociation curves at different pH values for *C. carnifex* (W. W. Burggren & B. R. McMahon, personal communication; Wood & Randall, 1981*b*).

Net flux rates (J_{net}) of each substance (e.g. X) between the crab and the external water pool were calculated as:

$$J_{net} = \frac{([X]_i - [X]_f) \times V}{t \times W}, \quad (2)$$

where i and f refer to initial and final concentrations (in μequiv ml^{-1} or μmol ml^{-1}), V is the volume of the external water pool (in ml), t is the elapsed time (in h) and W is the body weight (in kg). Thus net losses by the animal have a negative sign, net gains a positive sign. By reversing the i and f terms, the net titratable acidity flux was calculated from the titratable alkalinities. The sum of titratable acidity and ammonia fluxes gave the net flux of acidic equivalents ($J_{net}^{H^+}$; Maetz, 1973).

Rates of O_2 consumption ($\dot{M}_{O_2}$) were calculated as the sums of the rates from the air and water phases (if present). For each, an equation analogous to equation 2 was used, with the O_2 concentration calculated as the product of the measured P_{O_2} and the tabulated capacitance coefficient for the appropriate medium at the experimental temperature (Dejours, 1975). A similar approach was used for $\dot{M}_{CO_2}$, but here the CO_2 concentrations in water were measured directly, as the CO_2 capacitance of fresh water is not necessarily a constant (Dejours, 1975).

Data have been expressed as means ± 1 S.E.M., unless otherwise stated. The significance ($P \leq 0{\cdot}05$) of differences between means was assessed with a paired Student's two-tailed *t*-test, using each animal as its own control. One crab in the haemolymph series escaped after 108 h dehydration and was never seen again, while one in the gas exchange series died at 180 h, apparently from desiccation. Up until these times, the two crabs followed the general trends of the other animals, but their data have been omitted from means in view of the paired statistical design.

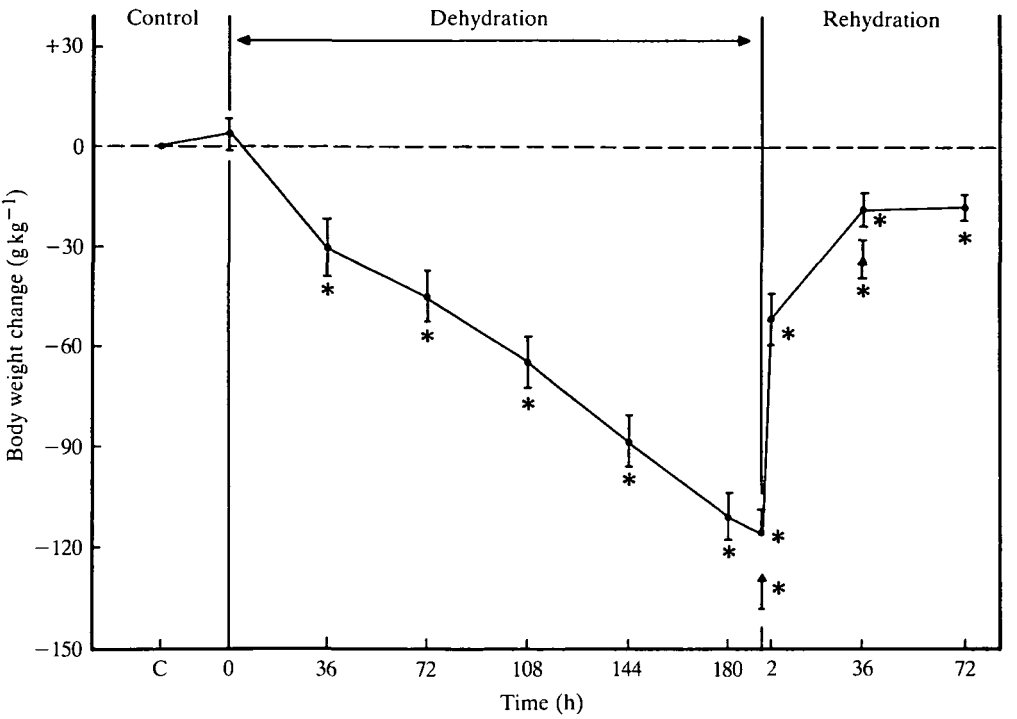

Fig. 1. Changes in body weight in *Cardisoma carnifex* during 192 h of dehydration followed by 72 h of rehydration. (●) Haemolymph series ($N = 8$); (▲) gas exchange series ($N = 7$), crabs not blood-sampled or handled. Means ± 1 S.E.M. * Significantly different ($P \leq 0{\cdot}05$) from control value.

RESULTS

Weight changes

Crabs lost weight at an initial rate of $0{\cdot}94\,g\,kg^{-1}\,h^{-1}$ (0–36 h) which declined to a stable value of $0{\cdot}55\,g\,kg^{-1}\,h^{-1}$ between 36 and 192 h dehydration (Fig. 1). By this time, the total weight loss averaged $116{\cdot}0 \pm 19{\cdot}4\,g\,kg^{-1}$ or ≈18% of the total body water pool ($620–655\,g\,kg^{-1}$; Cameron, 1981*b*; Harris & Kormanik, 1981). This loss was probably close to the lethal limit (cf. Burggren & McMahon, 1981; Harris & Kormanik, 1981); indeed one crab died at 180 h (weight loss = $153\,g\,kg^{-1}$). After only 2 h of rehydration, the animals had regained over half the lost weight and, by 36–72 h rehydration, the final weight stabilized approximately $20\,g\,kg^{-1}$ below the starting weight, the difference presumably reflecting metabolic weight loss over the 11-day experiment. Based on measured $\dot{M}_{O_2}$ (Fig. 2), a weight loss of $\approx 10\,g\,kg^{-1}$ is predicted from fat metabolism alone; undoubtedly some water would be lost with the fat. During the first few hours of rehydration the crabs flushed their branchial chambers with water almost continually, and a few exuded a yellowish fluid (gut fluid?) from the mouth. Weight changes measured at 192 h dehydration and 36 h rehydration in the gas exchange series, in which the animals were not handled and blood samples were not taken, were very similar to those in the haemolymph series (Fig. 1), indicating that these procedures did not accelerate the desiccation process.

Gas exchange

Under hydrated control conditions, $\dot{M}_{O_2}$ and $\dot{M}_{CO_2}$ were only about half those in a previous study (Wood & Randall, 1981*a*), possibly reflecting metabolism nearer the basal value (i.e. nearer resting conditions) or the use of fresh water rather than 50% sea water in the present investigation. However, the gas exchange ratio (R = 0·53) was again unusually low (Fig. 2). Dehydration caused marked reductions (≈55%) in

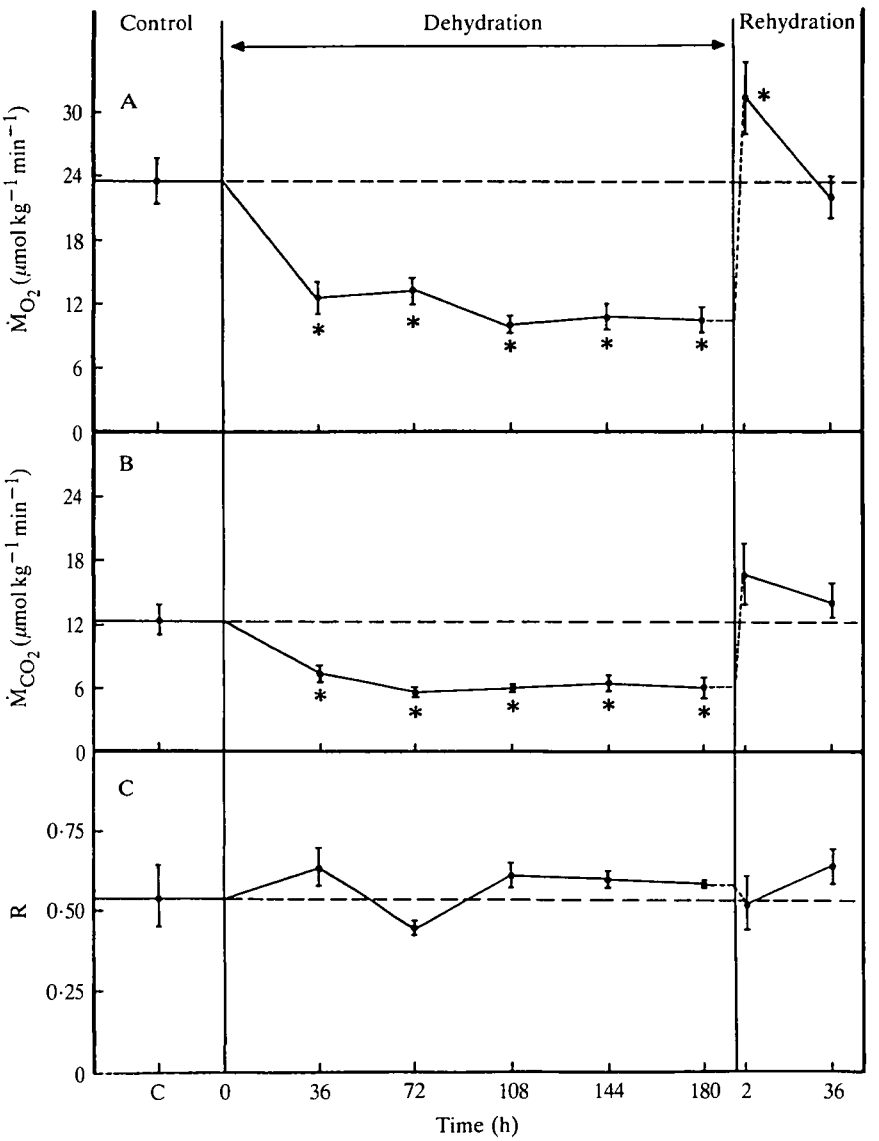

Fig. 2. Changes in (A) total O_2 consumption rate ($\dot{M}_{O_2}$); (B) total CO_2 excretion rate ($\dot{M}_{CO_2}$); and (C) the gas exchange ratio R($\dot{M}_{CO_2}/\dot{M}_{O_2}$) in *Cardisoma carnifex* during 192 h of dehydration (18% loss of body water) followed by 36 h of rehydration. Means ± 1 S.E.M. ($N = 7$). * Significantly different ($P \leq 0{\cdot}05$) from control value.

both $\dot{M}_{O_2}$ and $\dot{M}_{CO_2}$ which were established by 36 h and stable by 108 h. Within 2 h of rehydration, $\dot{M}_{O_2}$ and $\dot{M}_{CO_2}$ increased significantly to levels above the original control with a return to normal by 36 h. Throughout the experiment, $\dot{M}_{O_2}$ and $\dot{M}_{CO_2}$ changed proportionately, so R did not vary significantly. The crabs remained generally motionless during both the control and dehydration periods, so we do not think that the reduction in metabolism was due to a reduction in overt activity. However, upon rehydration, the crabs clearly became more active, so this may well explain the surge of metabolism above the control level at this time. Our respirometry system did not prevent gas equilibration between air and water phases; nevertheless it is interesting that while the fraction of $\dot{M}_{O_2}$ derived from water measurements was negligible ($\approx$2%), the comparable aquatic fraction of $\dot{M}_{CO_2}$ was both large and highly variable (−50% to +65% of the total).

There was no evidence that the fall in $\dot{M}_{O_2}$ during dehydration caused internal hypoxia. Haemolymph lactate levels remained very low and stable (Fig. 3B), while Pa_{O_2} actually increased (Fig. 3A). In view of the relatively high O_2 affinity of *C. carnifex* haemocyanin (P_{50} = 13·5 Torr under these conditions; W. W. Burggren & B. R. McMahon, personal communication; Wood & Randall, 1981*b*), these changes in Pa_{O_2} had minimal influence on arterial saturation which remained at 90–100%. Blood glucose levels fell significantly at 36 h, recovered, and then fell again at 180 h, but returned to normal during rehydration (Fig. 3C).

Haemolymph acid–base status

Haemolymph acid–base status during dehydration was characterized by alkalosis; pHa stabilized 0·05–0·1 units above the control level during the entire period of water deprivation (Fig. 4C). However, first Ca_{CO_2} (Fig. 4A) and later Pa_{CO_2} (Fig. 4B) progressively increased during dehydration. The initial effect (0–36 h) was a straight metabolic alkalosis with HCO_3^- accumulation at constant Pa_{CO_2} (Fig. 5). Thereafter, there occurred a partial respiratory compensation (Pa_{CO_2} build-up) of an increasing metabolic alkalosis. By 180 h, the haemolymph [HCO_3^-] had reached $\approx$ 28·5 mequiv l^{-1}, a value approximately 70% above control, reflecting a ΔH_h^+ of −13·1 mequiv l^{-1} (i.e. basic equivalent accumulation). Assuming an extracellular fluid volume (ECFV) of 195 ml kg^{-1} in *C. carnifex* (Kormanik & Harris, 1981; Harris & Kormanik, 1981), the total basic equivalent load in the haemolymph was 2555 μequiv kg^{-1}. By 36 h rehydration, virtually all of this base load has been removed from the haemolymph; pHa and Ca_{CO_2} returned to normal although Pa_{CO_2} remained slightly elevated (Figs 4, 5).

Haemolymph ionic status

Haemolymph [Na^+], [Cl^-], [Mg^{2+}], [K^+], total protein concentration (mainly haemocyanin) and osmolality all increased significantly during dehydration, and all except [K^+] returned to normal during rehydration (Figs 6, 7). The fall in protein level below control during rehydration presumably reflected sampling losses or starvation (Fig. 7B). Most increases in osmolytes during dehydration were in the range 8–15%, far smaller than the observed 70% rise in haemolymph [HCO_3^-].

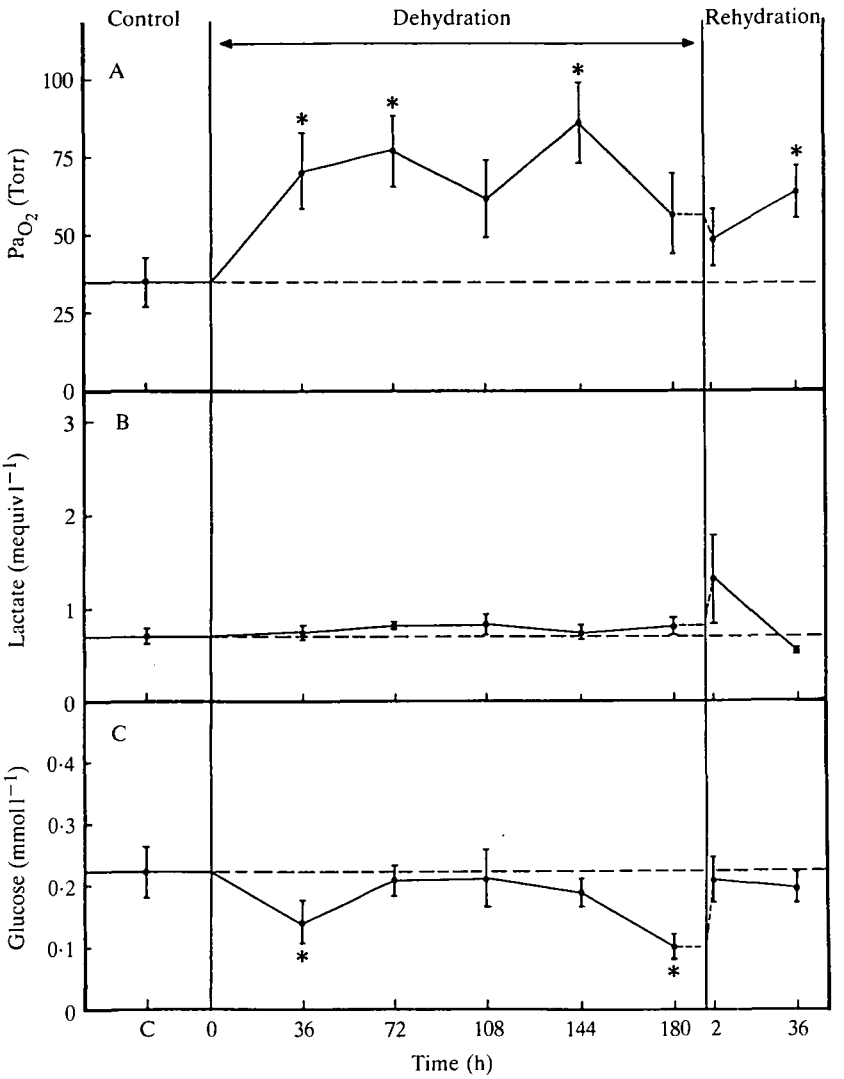

Fig. 3. Changes in (A) arterial haemolymph O_2 tension (Pa_{O_2}); (B) haemolymph lactate concentration; and (C) haemolymph glucose concentration in *Cardisoma carnifex* during 192 h of dehydration (18% loss of body water) followed by 36 h of rehydration. Means ± 1 S.E.M. ($N = 8$). * Significantly different ($P \leq 0{\cdot}05$) from control value.

Furthermore, with the single exception of $[Mg^{2+}]$ (29% increase, Fig. 6D), these elevations in haemolymph osmolytes, even after taking excretion (Table 2) into account, were smaller than anticipated had the measured 18% water loss been proportionately distributed between extracellular and intracellular spaces. Indeed, $[Cl^-]$ only increased at the final dehydration sample (Fig. 6B) and $[Ca^{2+}]$ showed no significant variation throughout the experiment (Fig. 6C). The latter observation tends to rule out $CaCO_3$ mobilization from the carapace (see Wood & Randall, 1981*b*) as the cause of the observed haemolymph metabolic alkalosis.

By the strong ion difference concept (Stewart, 1978), the increase in haemolymph [HCO_3^-] during metabolic alkalosis should be associated with an increased difference between strong cation and strong anion concentrations (SID). This was indeed the case; the mean SID, here calculated as [$Na^+ + Ca^{2+} + Mg^{2+} + K^+ - Cl^- -$ lactate] increased from 11·5 mequiv l^{-1} (control) to 26·2 mequiv l^{-1} (180 h dehydration) and then decreased to 24·8 mequiv l^{-1} (36 h rehydration). At these same times, mean HCO_3^- levels were 16·6, 28·5 and 19·2 mequiv l^{-1},

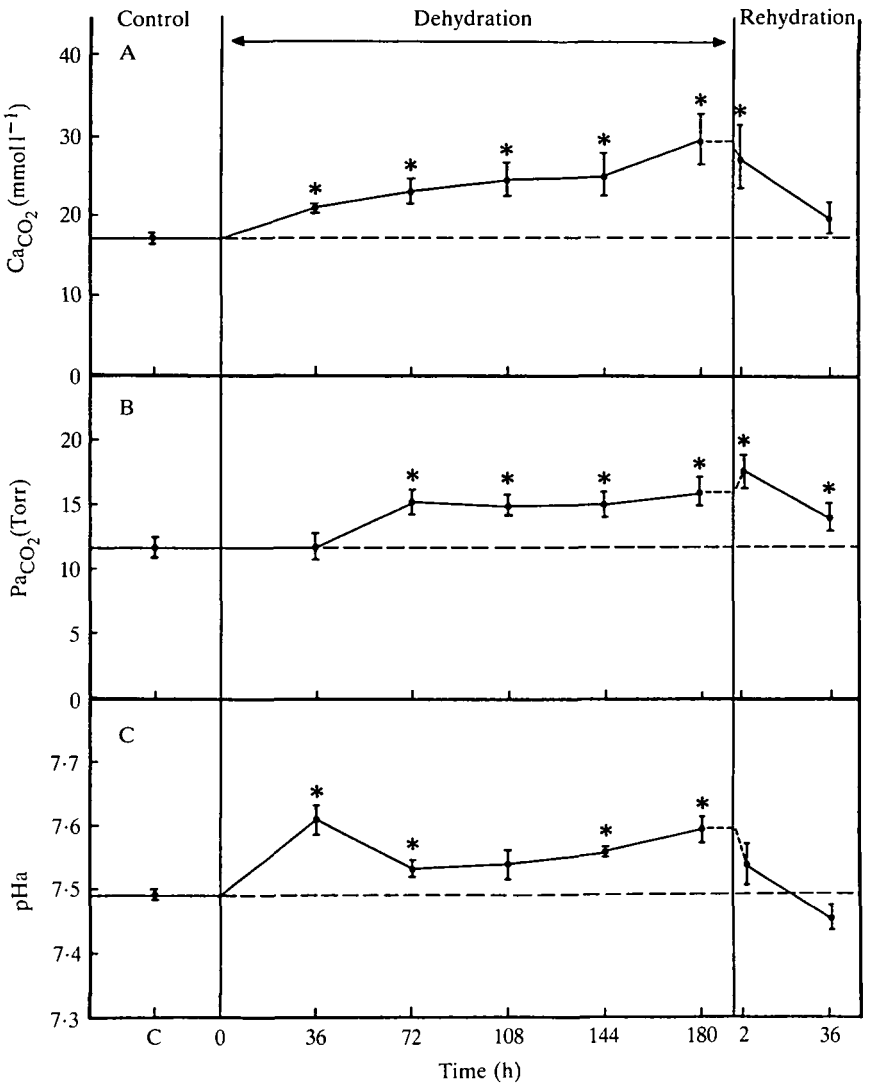

Fig. 4. Changes in the components of arterial haemolymph acid–base status in *Cardisoma carnifex* during 192 h of dehydration (18% loss of body water) followed by 36 h of rehydration. (A) total Co_2 concentration (Ca_{CO_2}); (B) CO_2 tension (Pa_{CO_2}); (C) arterial pH (pHa). Means ± 1 S.E.M. ($N = 8$). * Significantly different ($P \leq 0{\cdot}05$) from control value.

respectively. However, inspection of both mean and individual values indicated that the SID changes could not be correlated with changes in any one anion or cation, but rather reflected varying contributions from different ions at different times. The sum of all measured osmolytes (excluding protein) in $mmol\,l^{-1}$ was very close to the measured osmotic pressure in $mosmol\,kg^{-1}$; mean ratios (osmolytes/osmotic pressures) were virtually constant (range 1·00–1·03) over the experiment, suggesting that unmeasured substances (e.g. SO_4^{2-}) did not vary greatly.

Nitrogenous wastes

Under control hydrated conditions, ammonia was the major nitrogenous waste in the haemolymph at a mean level ($1{\cdot}26\,mmol\,l^{-1}$; Fig. 8A) approximately 10-fold greater than urea ($0{\cdot}14\,mmol\,l^{-1}$; Fig. 8B), while uric acid was undetectable (detection limit = $3\,\mu mol\,l^{-1}$). A single animal exhibited approximately 25-fold greater urea levels (Table 1) and therefore has been omitted from Fig. 8B. During dehydration, haemolymph ammonia doubled at 36 and 72 h, but then returned to normal for the remainder of the desiccation period (Fig. 8A). However, during rehydration, there was a very large but variable rise to a mean level greater than

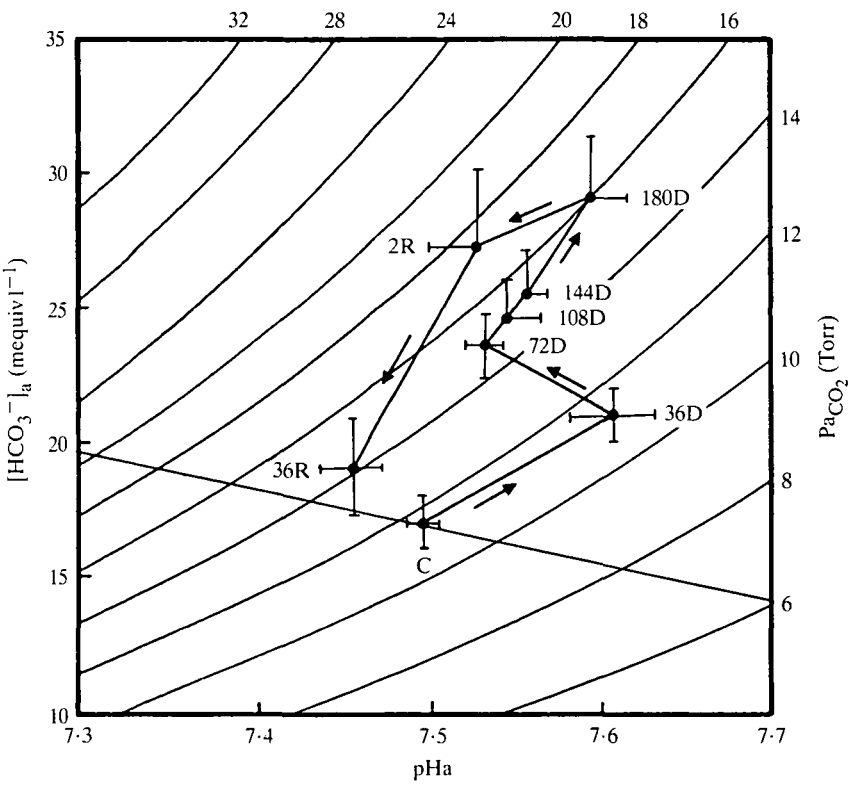

Fig. 5. Display of arterial haemolymph acid–base status on a pH–HCO_3^- diagram in *Cardisoma carnifex* during 192 h of dehydration (18% loss of body water; D) followed by 36 h of rehydration (R); C, control. Transverse line represents the non-HCO_3^- buffer line with slope of 12·1 slykes from Wood & Randall (1981*b*). Means ± 1 S.E.M. ($N = 8$).

7 mmol l^{-1} at 36 h. This pattern of ammonia changes clearly did not explain the observed metabolic alkalosis in the haemolymph (Figs 4, 5), for during dehydration ammonia returned to normal while basic equivalent accumulation progressed, and

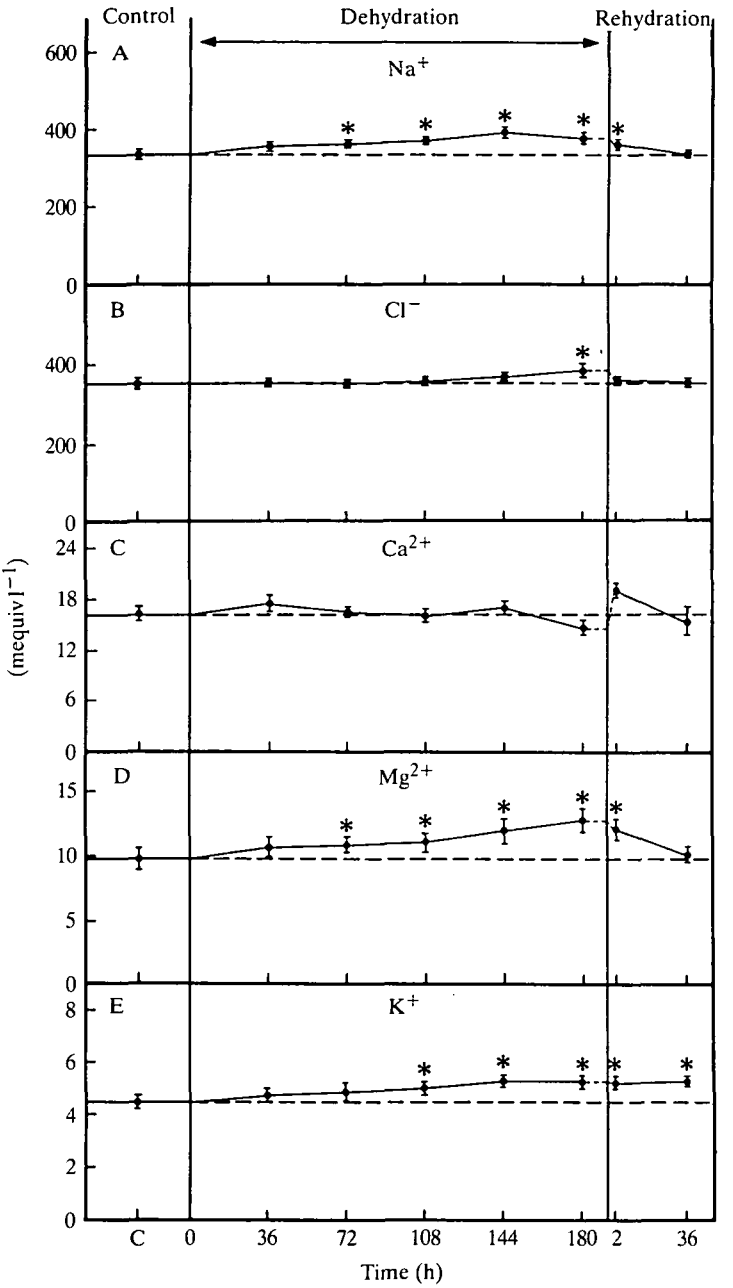

Fig. 6. Changes in haemolymph ionic status in *Cardisoma carnifex* during 192 h of dehydration (18% loss of body water) followed by 36 h of rehydration. (A) Sodium, (B) chloride, (C) calcium, (D) magnesium and (E) potassium. Means ± 1 S.E.M. ($N = 8$). * Significantly different ($P \leq 0{\cdot}05$) from control value.

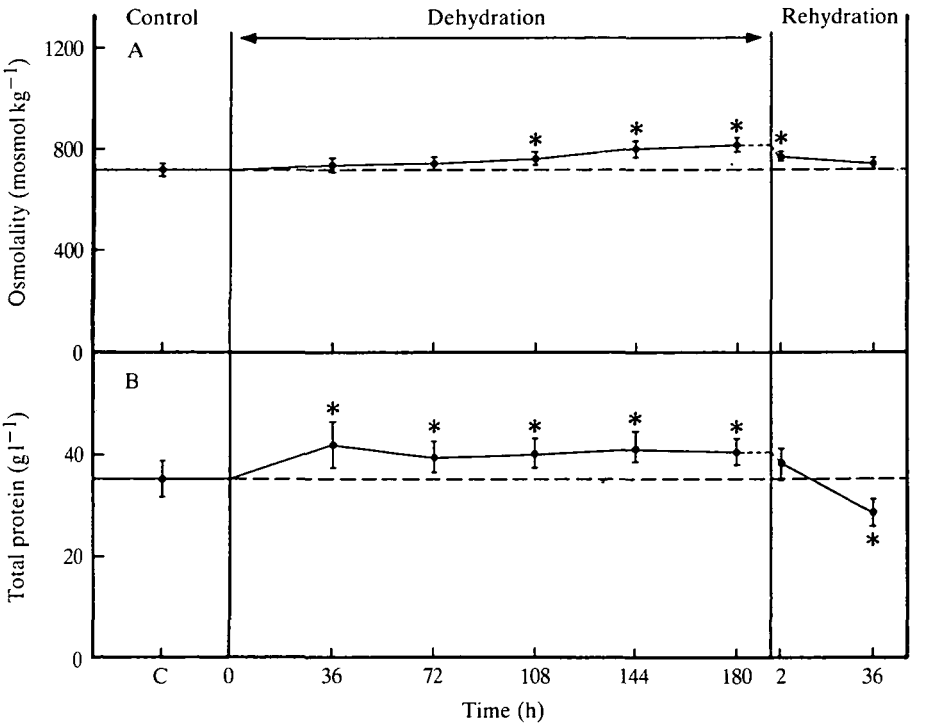

Fig. 7. Changes in (A) haemolymph osmolality and (B) total protein concentration in the haemolymph in *Cardisoma carnifex* during 192 h of dehydration (18% loss of body water) followed by 36 h of rehydration. Means ± 1 S.E.M. ($N = 8$). *Significantly different ($P \leq 0{\cdot}05$) from control value.

later greatly increased during rehydration while basic equivalents were removed from the haemolymph. Urea levels varied considerably during the experiment but showed no significant changes overall (Fig. 8B). A single crab, the one with the unusually high urea levels, exhibited significant uric acid accumulation in the blood during dehydration and removal during rehydration (Table 1). However even at its peak, uric acid accounted for less than 26% of the total nitrogen relative to the high levels of ammonia and urea in this animal. Uric acid remained below detection limits throughout the experiment in the other eight crabs.

Ammonia was also the major nitrogenous waste excreted into the external water under control conditions (Fig. 9A), although a very low urea efflux occurred in all animals (Fig. 9B). Uric acid excretion was not detected in any crab (detection limit in water = 0·5 μmol l^{-1}). No attempt was made to check whether ammonia excretion occurred by direct volatilization to the atmosphere during dehydration in the present experiments, though an earlier pilot study indicated that *C. carnifex* does not carry out this process (Wood & Boutilier, 1985). Washings from the dry bucket after 8 days dehydration revealed negligible ammonium and no uric acid precipitation, though the accumulated urea in the containers was equivalent to about 12 h excretion of urea under control conditions (Table 2). Upon rehydration after 192 h desiccation, ammonia efflux exhibited an immediate 3- to 4-fold increase above the relatively low

control level, an effect which persisted for at least 53 h (Fig. 9A). This was presumably associated with the very high haemolymph ammonia concentrations at this time (Fig. 8A). Urea excretion also increased markedly, but only during the 2-h period immediately following rehydration (Fig. 9B). Again uric acid excretion was not detected. Rather surprisingly, the crab with the abnormal haemolymph

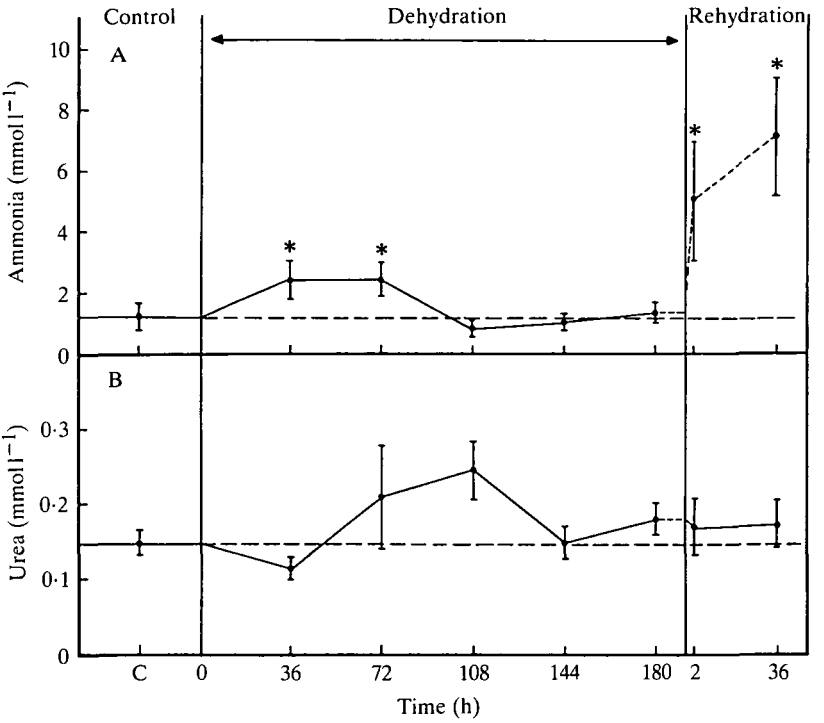

Fig. 8. Changes in the concentrations of (A) total ammonia ($N = 8$) and (B) urea ($N = 7$) in the haemolymph of *Cardisoma carnifex* during 192 h of dehydration (18% loss of body water) followed by 36 h of rehydration. Means ± 1 S.E.M. * Significantly different ($P \leq 0{\cdot}05$) from control level.

Table 1. *Nitrogenous wastes ($\mu mol\, l^{-1}$) in the haemolymph of a single* Cardisoma carnifex *exhibiting uric acid production during dehydration and rehydration*

	Ammonia	Urea	Uric acid
Control	1600	3870	ND
Dehydration (36 h)	1910	1230	3
Dehydration (72 h)	5120	—	27
Dehydration (108 h)	1450	3320	35
Dehydration (144 h)	3170	1040	82
Dehydration (180 h)	810	1640	351
Rehydration (2 h)	14310	2780	62
Rehydration (36 h)	19590	1970	21

ND, not detectable.

composition (Table 1) exhibited normal excretion rates of ammonia and urea and no uric acid efflux.

Ionic and acidic equivalent exchange

Under control conditions, crabs were not in steady state but rather in net positive balance for Na^+ and Cl^- (Fig. 10A,B), taking up both ions at approximately

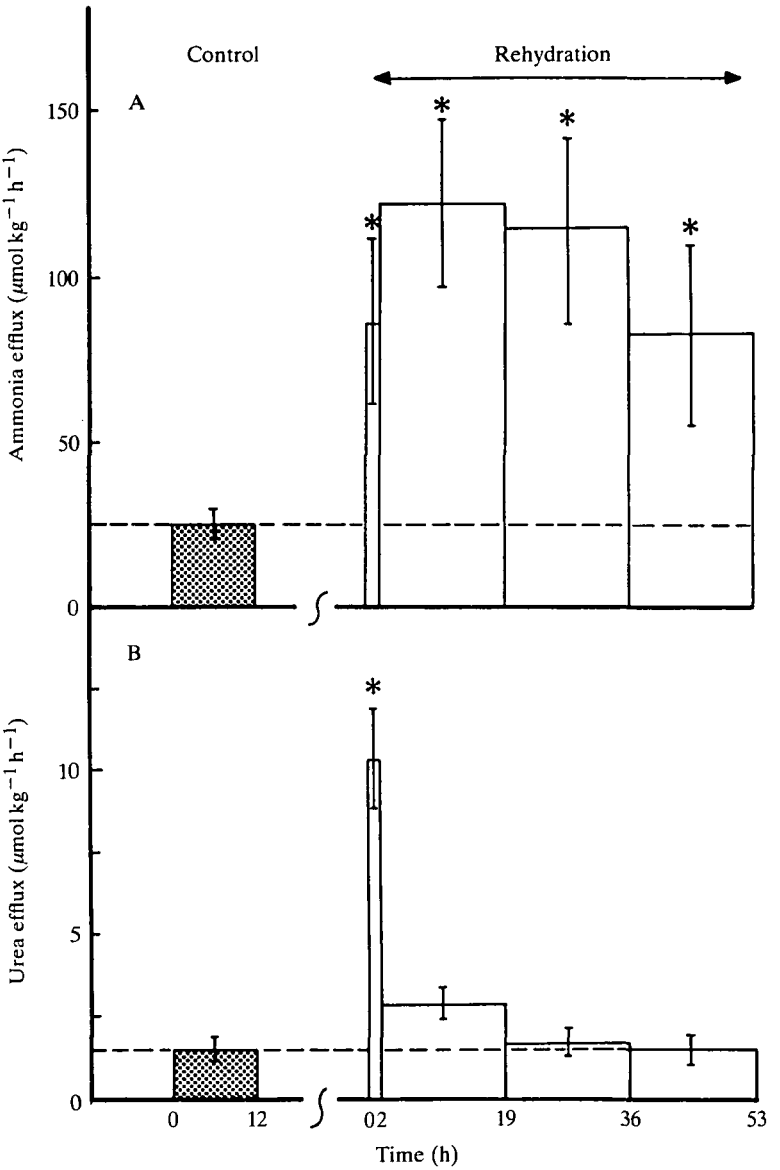

Fig. 9. The net efflux of (A) total ammonia and (B) urea into the environmental water from *Cardisoma carnifex* under control conditions (stippled) and during 53 h of rehydration after a 192-h period of dehydration (18% loss of body water). Means ± 1 S.E.M. ($N = 8$). * Significantly different ($P \leq 0{\cdot}05$) from control value.

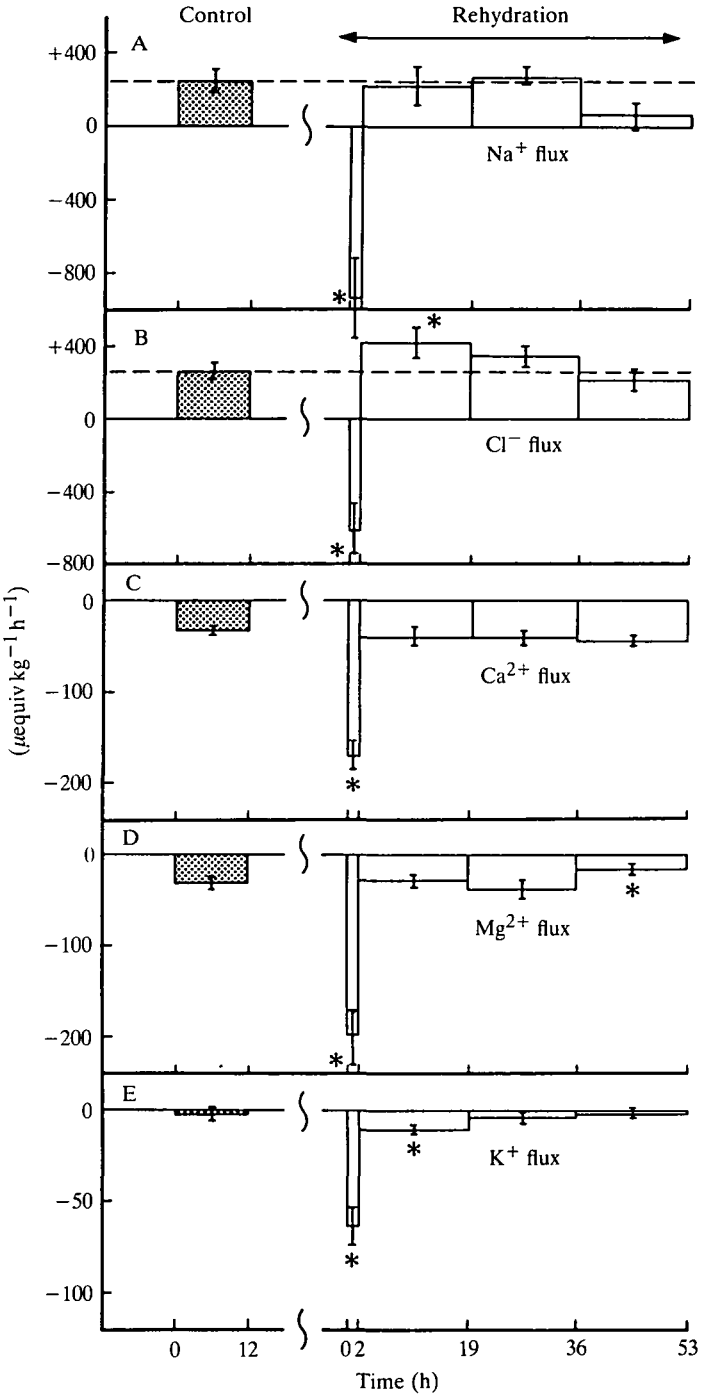

Fig. 10. The net fluxes of (A) sodium, (B) chloride, (C) calcium, (D) magnesium and (E) potassium between *Cardisoma carnifex* and its environmental water under control conditions (stippled) and during 53 h of rehydration after a 192-h period of dehydration (18% loss of body water). Positive fluxes represent uptakes by the crabs, negative values represent net losses. Means ± 1 S.E.M. ($N = 8$). * Significantly different ($P \leq 0{\cdot}05$) from control value.

+250 μequiv kg^{-1} h^{-1} from the external fresh water, and net negative balance for Ca^{2+} and Mg^{2+} (Fig. 10C,D), losing both at approximately −30 μequiv kg^{-1} h^{-1}. There was no significant net K^+ flux (Fig. 10E). Small amounts of all ions were lost to the dry buckets over 8 days of dehydration (Table 2). These losses were equivalent to haemolymph clearances of approximately 1 ml kg^{-1} for Na^+ and Cl^-, 3–5 ml kg^{-1} for K^+ and Mg^{2+}, and 9 ml kg^{-1} for Ca^{2+} (cf. Fig. 6). Nevertheless, this total Ca^{2+} efflux was negligible (< 6 %) relative to the accumulated load of basic equivalents (2555 μequiv kg^{-1}) in the haemolymph during desiccation, again arguing against $CaCO_3$ mobilization from the carapace as the source of the latter. During the first 2 h of rehydration, there were large net losses of all ions into the external medium (Fig. 10), effects which could have resulted from the flushing of highly concentrated fluid or precipitates out of the branchial chambers, gut or renal systems. Over the subsequent 51 h, net Na^+ uptake and Ca^{2+} loss rates remained at the control levels (Fig. 10A,C). However, net Cl^- uptake and K^+ loss rates were stimulated at the 2–19 h rehydration period (Fig. 10 B,E), while Mg^{2+} losses were significantly reduced during the 36–53 h period (Fig. 10D).

$J^{H^+}_{net}$, calculated as the sum of the titratable acidity (not shown) and ammonia fluxes (Fig. 9A), signs considered, was significantly positive under control conditions (Fig. 11A); the crabs took up acidic equivalents and/or excreted basic equivalents at a net rate ≈120 μequiv kg^{-1} h^{-1}. Upon rehydration, $J^{H^+}_{net}$ increased markedly during the first 2 h and then remained elevated at a level averaging ≈160 % of the original control until 53 h (Fig. 11A). It should be emphasized that this apparent base excretion was in addition to any ammonia efflux which occurred as NH_3, for while NH_3 certainly has an alkalinizing influence, its movement is considered neutral with respect to acid–base balance, both theoretically and in the $J^{H^+}_{net}$ calculation. Relative to the control rate, the increased $J^{H^+}_{net}$ during 53 h rehydration accounted for an apparent base excretion of 3945 μequiv kg^{-1}, more than sufficient to explain the

Table 2. *Total excretions of nitrogenous wastes and inorganic electrolytes (μequiv kg^{-1}) recovered from the containers of* Cardisoma carnifex *after 192 h dehydration*

	Excretion	% of haemolymph content*
Ammonia	12·3 ± 7·3	—
Urea	12·7 ± 6·1	—
Uric acid	ND	—
Na^+	350·0 ± 91·0	0·54
Cl^-	454·7 ± 146·5	0·66
K^+	14·8 ± 2·9	1·69
Ca^{2+}	143·6 ± 3·7	4·54
Mg^{2+}	48·4 ± 11·7	2·51

$\bar{X}$ ± 1 S.E.M., $N = 8$.

*Calculated assuming the control concentration values of Fig. 6 and an ECFV of 195 ml kg^{-1} (Harris & Kormanik, 1981).

ND, not detectable.

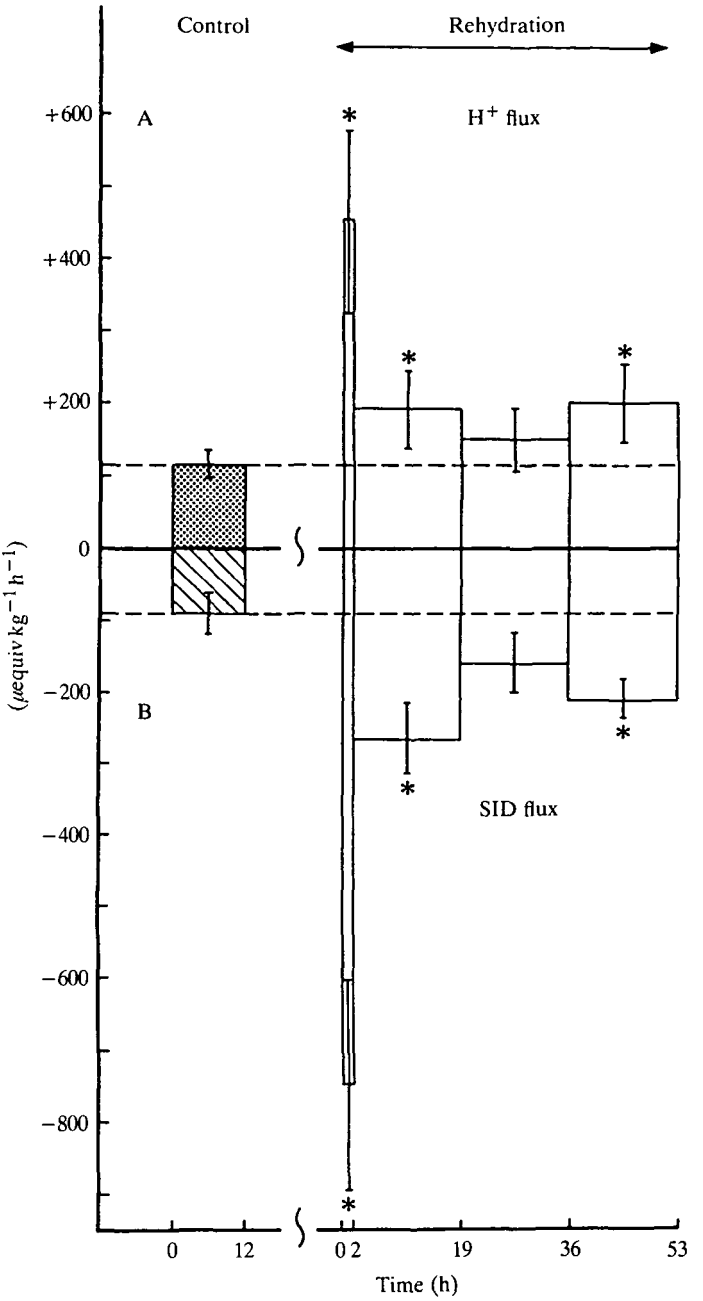

Fig. 11. (A) The net fluxes of acidic equivalents ($J_{net}^{H^+}$) and (B) the net strong ion difference (SID) fluxes, here calculated as $[Na^+ + K^+ + Ca^{2+} + Mg^{2+} - Cl^-]$ fluxes, between *Cardisoma carnifex* and its environmental water under control conditions (stippled, cross-hatched) and during 53 h of rehydration after a 192-h period of dehydration (18% loss of body water). Positive fluxes represent net uptakes by the crabs, negative value represent net losses. Means ± 1 S.E.M. ($N = 8$). * Significantly different ($P \leq 0{\cdot}05$) from control value.

observed removal from the haemolymph of approx. 2555 μequiv kg^{-1} basic equivalents which had accumulated during dehydration.

Net $[Na^+ + K^+ + Ca^{2+} + Mg^{2+} - Cl^-]$ flux was calculated as an index of the strong anion minus strong cation, or SID, flux (Stewart, 1978; Fig. 11B). This closely mirrored the changes in $J_{net}^{H^+}$ (Fig. 11A) and was also in good quantitative agreement with $J_{net}^{H^+}$, except during the first 2 h of rehydration. This is in accord with the constraints of electrical neutrality, and suggests that the fluxes of most quantitatively important electrolytes (except perhaps SO_4^{2-}) have been accounted for. However, inspection of the data from both individual crabs and the summarized values of Fig. 10 clearly showed that the trends in SID flux reflected varying contributions from different ions at different times. As with haemolymph SID, changes in any one particular anion or cation did not predominate.

DISCUSSION

Rate of dehydration

After a slightly higher initial weight loss (0·94 g kg^{-1} h^{-1}), perhaps reflecting the evaporation of branchial chamber water over the first 36 h, *C. carnifex* dehydrated at only a very slow rate (0·55 g kg^{-1} h^{-1}) during continued water deprivation (Fig. 1). This may be compared with previously measured rates of 1·5–5·0 g kg^{-1} h^{-1} in the same species and other gecarcinids (Gross, Lasiewski, Dennis & Rudy, 1966; Bliss, 1968; Herreid, 1969; Harris, 1977; Burggren & McMahon, 1981; Harris & Kormanik, 1981), and 0·65–1·07 g kg^{-1} h^{-1} in terrestrial hermit crabs, where the molluscan shell greatly inhibits water loss (McMahon & Burggren, 1979; Burggren & McMahon, 1981; Wheatly *et al.* 1984). The low rates seen here probably reflected the different experimental conditions of the present investigation; relative humidity, while not assayed, was undoubtedly greater than 90% in the covered containers (*vs* 25–90% in other studies), convection currents were virtually non-existent, and disturbance to the animals was minimal except at times of weighing. The crabs otherwise remained almost motionless in their containers throughout dehydration. Upon rehydration, restoration of lost water was very rapid (Fig. 1), in accord with previous studies, and the animals became more active. These experimental conditions may duplicate those in the deep, blind-ended burrows (Wood & Boutilier, 1985), when the water table rises and falls during periods of rain and drought. However, we cannot prove that this is the case, as the only dry burrow which we found was apparently disused.

Gas exchange and metabolism

The sustained 55% decrease in $\dot{M}_{O_2}$ throughout dehydration (Fig. 2A) is similar to that seen in the intertidal crab *Pachygrapsus* (Gross, 1955) and the 'fresh water' land crab *Holthuisana* (MacMillen & Greenaway, 1978). At least in *Cardisoma*, this reduction in $\dot{M}_{O_2}$ did not cause internal hypoxia (Fig. 3) nor did it lead to a change in the respiratory quotient of the animal, as $\dot{M}_{CO_2}$ was similarly depressed (Fig. 2B). These data, taken together with our measurement of a marked decline in branchial

chamber ventilation during dehydration (R. G. Boutilier & C. M. Wood, in preparation), are a general indication of a depression in metabolic rate. In accord with MacMillen & Greenaway (1978), we interpret this as a water- and energy-conserving mechanism allowing the animals to withstand prolonged periods of drought in their burrows.

The gas exchange ratio, R, remained unusually low (0·5–0·6; Fig. 2C) but constant during dehydration, indicating that the presence of branchial water was not obligatory for CO_2 excretion, at least at the reduced levels of CO_2 production occurring at this time. Although some CO_2 retention in the haemolymph (associated with metabolic alkalosis, Fig. 4A) did occur during dehydration, this amounted to only approx. 3 % of the total $\dot{M}_{CO_2}$ over the 8-day desiccation period. Certainly, the respiratory exchange quotient was lower than that usually considered to be the theoretical minimum for standard aerobic respiration. Ideas about the underlying metabolic processes are developed below. Such processes must also have been in effect throughout dehydration since R values remained at these unusually low levels throughout the dehydration period.

Acid–base regulation

In contrast to previous studies on *C. carnifex* and other land crabs, where the animals developed combined metabolic and respiratory acidoses during dehydration (Burggren & McMahon, 1981; Wheatly *et al.* 1984), our animals exhibited a small increase in blood pH which thereafter remained relatively stable (Figs 4, 5). The differences between this and other studies were probably related to the much slower rate of dehydration which allowed our animals to better regulate ECFV and composition (see below) and thereby minimize disturbances in blood gas transport and acid–base regulation. The primary response was a progressively developing metabolic alkalosis (accumulation of basic equivalents) followed by a partially compensating respiratory acidosis (Pa_{CO_2} build-up) (Figs 4, 5). While the Pa_{CO_2} retention is explained by the observed hypoventilation during dehydration (R. G. Boutilier & C. M. Wood, in preparation), the cause of the metabolic alkalosis is less certain. Nevertheless, the present data rule out several possibilities. These include: decrease of ECFV resulting in HCO_3^- elevation by haemoconcentration ('contraction alkalaemia', Hills, 1973) (compare Fig. 5 with Figs 6 and 7); $CaCO_3$ mobilization from the carapace (Fig. 6C; Table 2); entrainment by disturbances of one particular strong anion or cation (Figs 6, 10); and ammonia accumulation in the haemolymph (Fig. 8A). We can, however, put forward one reasonable explanation on the basis of our data. Under control hydrated conditions, *C. carnifex* exhibited a significantly positive J_{net}^{H+} with the environmental water (Fig. 11A). This uptake of acidic equivalents (and/or excretion of basic equivalents) was presumably blocked by dehydration. If we assume that, like $\dot{M}_{O_2}$ and $\dot{M}_{CO_2}$ (Fig. 2), the metabolic process which necessitates positive $J_{net}^{H^+}$ continued during dehydration at 45 % of the control rate, then approx. 9500 μequiv kg^{-1} of basic equivalents would have been accumulated by 180 h. The estimated haemolymph load was 2555 μequiv kg^{-1} at this time. The difference could well have been stored in the much larger intracellular

fluid volume (ICFV) (Cameron, 1981*b*) or carapace compartments; the latter represents a massive sink for basic equivalents (Wood & Cameron, 1985; Cameron & Wood, 1985). Indeed, the elevated $J_{net}^{H^+}$ during rehydration was more than enough to explain the correction of haemolymph acid–base status over this period. An advantage of this metabolic base accumulation during desiccation would be to neutralize respiratory acidosis, for the burrows in which the animals would probably undergo dehydration in the wild are markedly hypercapnic ($P_{I_{CO_2}} \approx 16$ Torr; Wood & Boutilier, 1985). We suggest that the cause of continuing metabolic base production and excretion may in some way be related to the herbivorous feeding strategy of *C. carnifex*, for the metabolism of most herbivorous diets is strongly base producing (Hills, 1973; Atkinson & Camien, 1982). Unfortunately, it is not clear whether this process would continue for the starvation period employed in the present experiments.

These acid–base data certainly do not support an earlier hypothesis (Wood & Randall, 1981*a*; Randall & Wood, 1981) that the unusually low R value of *C. carnifex* results from fixation of some respiratory CO_2 for carapace growth. This scheme would necessitate the *excretion* of two hydrogen ions for every CO_2 stored as CO_3^{2-} (Cameron & Wood, 1985), rather than the observed *uptake* of H^+. Additional evidence against the previous idea includes the persistent negative calcium balance throughout the experiment (Fig. 10C), and the fact that carapace mineralization is probably not a continuous process, but rather occurs rapidly in the post-moult stage only (Greenaway, 1985). Having discounted this hypothesis, we are unfortunately at a loss to explain either the abnormally low R value, or the apparent persistence of metabolic base production in the absence of feeding. Further metabolic studies are clearly required.

Ionoregulation

While Na^+ and Cl^- levels in the haemolymph were approximately normal, Ca^{2+}, Mg^{2+} and K^+ concentrations were 20–50 % below 'field' values, presumably because of the week's holding period in fresh water plus starvation prior to experimentation (Wood & Boutilier, 1985).

The concentrations of most haemolymph osmolytes (Figs 6, 7) increased less than the 18 % loss of body water, suggesting preferential regulation of the extracellular fluid during dehydration. This is in agreement with Harris & Kormanik (1981), who found similar $[Na^+]$, $[Cl^-]$ and osmotic pressure changes in *C. carnifex* and *Gecarcoidea lalandii* dehydrated to the same extent. They also showed, by direct measurement, that the ECFV was maintained constant. In contrast, Burggren & McMahon (1981) reported 25–75 % increases in haemolymph osmolytes in the same two species dehydrated to the same weight loss. Again, this probably reflects differences in the rate of dehydration and/or experimental conditions between the studies.

When kept on sand dampened with 80 % sea water, *C. carnifex* produced urine at approx. $1{\cdot}3\ ml\ kg^{-1}\ h^{-1}$ (Kormanik & Harris, 1981). Urine production was probably even higher under the freshwater hydration conditions of the present study. Had this

rate of urine excretion continued during water deprivation, crabs would have lost weight at least 2·5-fold faster than observed (Fig. 1). Furthermore, the observed losses of electrolytes to the dry containers amounted to total haemolymph clearances of only 1–9 ml kg^{-1} over 192 h (Table 2). Clearly either the urine was reabsorbed (e.g. *via* mouth or branchial chambers; cf. Wolcott & Wolcott, 1985), or urine production greatly decreased during dehydration. In *Gecarcinus lateralis*, Harris (1977) has presented evidence against the former possibility, and in *C. carnifex* and *Gecarcoidea lalandii*, Harris & Kormanik (1981) have shown that inulin clearance from the haemolymph ceases during dehydration, supporting the latter idea.

The present flux measurements (Figs 10, 11) are in agreement with our earlier findings that *C. carnifex* exhibits positive $J_{net}^{H^+}$ under control conditions, shows excellent correlation between $J_{net}^{H^+}$ and SID fluxes, is capable of net Na^+ and Cl^- uptake from fresh water, and is in slightly negative Ca^{2+} and Mg^{2+} balance in the absence of feeding (Wood & Boutilier, 1985). Furthermore, in that study, during rehydration after much milder dehydration (48 h, 40 g kg^{-1} water loss), increased net Cl^- uptake similarly occurred. This was due to increased unidirectional Cl^- influx, presumably at the gills; the same explanation may apply in the present study (Fig. 10B). However, mildly dehydrated crabs also showed increased net Na^+ uptake and no change in $J_{net}^{H^+}$, in contrast to the present animals, differences probably resulting from the degree of desiccation and the constraints of electrical neutrality in relation to acid–base exchange.

Nitrogen metabolism

After an initial doubling during the first 72 h of dehydration, haemolymph ammonia returned to control levels during continued water deprivation in *C. carnifex* (Fig. 8A). Negligible ammonium was recovered from the dry containers at the end of the experiment (Table 2), and alternative end products (urea, uric acid; Fig. 8B; Tables 1, 2) were not accumulated or excreted to any great extent. The prominent uric acid deposits which we had observed in a different population of this species in Palau (Wood & Randall, 1981*a*; Henry & Cameron, 1981) were never seen in the Moorea animals, before or after dehydration. This must mean either that ammonia excretion was achieved by direct volatilization to the atmosphere or that *net* ammonia production ceased during dehydration. We favour the latter explanation since in our earlier study, we were unable to detect any volatilization up to 48 h dehydration (Wood & Boutilier, 1985). The $\dot{M}_{O_2}$ and $\dot{M}_{CO_2}$ data (Fig. 2) indicate a general metabolic depression, suggesting a decrease in ammoniagenesis. Furthermore, the large sustained increases in both the levels of ammonia in the haemolymph and the excretion of ammonia during rehydration (Figs 8A, 9A) suggest that ammonia production was turned on again at this time. Indeed, over 53 h rehydration, the ammonia excretion in excess of control levels ($\approx$4310 mmol kg^{-1}) was almost sufficient to compensate for the 'missing' ammonia excretion ($\approx$4700 mmol kg^{-1}) during 192 h of dehydration. Similar changes were seen after mild, 48 h dehydration in *C. carnifex* (Wood & Boutilier, 1985). The processes involved in switching ammonia production off and on in land crabs are unknown. However, comparable

effects have been seen in mammalian hibernators and are associated with an ability to recycle nitrogen through urea back into amino acids *via* micro-organisms in the gut (Hochachka & Somero, 1984); it is therefore interesting that *C. carnifex* produced and excreted small amounts of urea (Figs 8B, 9B; Table 1).

This is only one of the many aspects of nitrogen metabolism in *Cardisoma* which deserve further investigation. For example, total nitrogenous waste excretion rates in both *C. carnifex* and *C. guanhumi* (Fig. 9A; Horne, 1968; Gifford, 1968; Wood & Boutilier, 1985) are less than 20% of those in a herbivorous vertebrate of comparable size (e.g. the carp, Vellas & Sefarty, 1974). On the other hand, the animal can tolerate very high blood ammonia levels (e.g. 7 mmol l^{-1} during dehydration, Fig. 8A) which would almost certainly be very toxic in vertebrates (Warren, 1958; Hillaby & Randall, 1979). Of more than 30 animals examined in the Moorea population, only one crab showed unequivocal evidence of uric acid production (Table 1), despite the fact that this compound was routinely found in the burrows (Wood & Boutilier, 1985). To further complicate matters, the same species in Palau routinely exhibited higher levels of uric acid than ammonia in the haemolymph, and no evidence of urea (Henry & Cameron, 1981)! Similar discrepancies occur between the findings of Horne (1968) and Gifford (1968) on *C. guanhumi*. Clearly, there remains much to learn about nitrogen metabolism in this most clever animal.

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