THE JOURNAL OF EXPERIMENTAL ZOOLOGY 218:53-64 (1981)

Hemolymph Oxygen Transport, Acid-Base Status, and Hydromineral Regulation During Dehydration in Three Terrestrial Crabs, *Cardisoma, Birgus,* and *Coenobita*

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ABSTRACT Hemolymph oxygen transport, acid-base status, and hydromineral regulation have been measured at 28–30°C in *Cardisoma carnifex, Birgus latro,* and *Coenobita brevimanus* in their normal hydrated state, following a period of severe dehydration, and after a subsequent 24-hour period of rehydration.

Cardisoma and *Birgus* tolerated a maximum loss of body water of 20%, which developed in 3–4 days without water, and resulted in a 25–30% increase in hemolymph osmolality. Ion concentrations also increased considerably, with the largest percentage increases in ion concentration occurring for Mg^{++}, Ca^{++}, and K^{+}.

A metabolic acidosis and a resultant fall in HCO_3^- developed in *Cardisoma* and *Birgus*, but PCO_2 showed no large changes, suggesting little respiratory compensation. Oxygenation of postbranchial hemolymph was not affected during dehydration, since P_{aO_2} and hemolymph O_2 capacity increased, the latter because of a hemoconcentration. Within 24 hours of rehydration, a restoration of the original levels of hemolymph ions and osmolality occurred, and a nearly complete return to normal acid-base status developed.

Coenobita was distinctive, since dehydration to the maximum tolerated 28% loss of body water appeared to be accompanied by a closer regulation of hemolymph ion levels. Only protein increased, which probably accounted for some of the rise in hemolymph osmolality. However, hemolymph O_2 capacity fell, and that, in conjunction with a significant reduction in P_{aO_2}, suggested a more severe disruption of O_2 transport than in either *Cardisoma* or *Birgus*. The metabolic acidosis and the fall in HCO_3^- during dehydration were also more profound, and *Coenobita* showed the least recovery of these three species after 24 hours of rehydration.

The colonization of the supralittoral habitat by anomuran and brachyuran crabs has required considerable morphological, physiological and behavioral modification. Two biological processes in particular—hydromineral regulation and gas exchange—have been under intense selective pressure in such Crustacea, in part because of the radically different nature of a gaseous as opposed to an aquatic respiratory medium, and also because of the inability to carry out osmoregulation and ion regulation in air alone. Since the gills have a major function not only in gas exchange but also in ion regulation, it is not surprising that these organs should have shown modification with the evolution of a terrestrial lifestyle (Harms, '32; Edney, '60; Bliss, '68; Taylor and Butler, '78), or even be largely superceded by other organs serving for respiration (Harms, '32) or hydromineral regulation (Gross, '64; Harris, '77; Harris and Kormanik, '81).

It is important to emphasize that, in many respects, the processes of respiration and hydromineral balance even in terrestrial decapods are inseparable, as adjustments in one process may require compensatory changes in the other. For example, changes in inorganic ions in the hemolymph may profoundly influence the oxygen-carrying properties of the blood (Larimer and Riggs, '64; Truchot, '75;

0022-104X/81/2181-0053$03.50

Weiland and Mangum, '75; Mangum, '81, for review). Furthermore, imbalance in body water and ion levels may cause metabolic adjustments, resulting in the production of acidic end products, which could also exert a considerable influence over hemolymph oxygen and carbon dioxide transport.

This study reports on interactions between hemolymph water and ion content and respiration and acid-base balance in terrestrial decapods during dehydration and subsequent recovery. One species of brachyuran crab (*Cardisoma carnifex*) and two species of anomurans (*Birgus latro, Coenobita brevimanus*), all from tropical supralittoral habitats, have been examined (see other articles in this volume for further details of their biology).

MATERIALS AND METHODS

All experimental animals were collected at night or dawn, above the high tide line on small islets in the Palau Island group of the Western Carolines. The crabs were transferred on board the R.V. Alpha Helix, where they were maintained prior to experimentation. Crabs of all three genera were kept initially at 28–30°C in humid (r.h. 78–90) terraria containing fresh leaf litter, and were initially given free access to both fresh water and 100% seawater. *Coenobita* and *Birgus* preferred fresh water, which was subsequently provided ad libitum. Shredded coconut and other vegetation was supplied, upon which the crabs fed frequently. Mean daily temperature and humidity profiles recorded on the deck of the Alpha Helix, but assumed to be representative of surface changes in the natural environment, are presented elsewhere (McMahon and Burggren, '81).

Hemolymph sampling and analysis

All hemolymph sampling was on unanaesthetised crabs. Prebranchial hemolymph was sampled through the arthrodial membrane at the base of the left or right cheliped in all three genera. Postbranchial hemolymph was sampled from a dorsal sinus posterior and lateral to the heart. Postbranchial samples were taken first, and the initial sampling for hemolymph gases (see below) was always completed within 1 minute of the first disturbance of the animal. McMahon and Burggren ('79) give details of hemolymph sampling techniques used for these terrestrial decapods.

Approximately 200 μl of hemolymph was drawn into a 250-μl Hamilton gas-tight syringe and analysed for P_{CO_2}, P_{O_2}, and pH in either an Instrumentation Laboratories μ13 or Radiometer PHM73 blood gas analyser. Oxygen content was measured on 40 μl of hemolymph with a Lex-O_2-Con analyzer (McMahon et al. '78), and total CO_2 content of a separate 40 μl was measured by the method of Cameron ('71). In vivo concentration of bicarbonate (plus carbonate) for prebranchial hemolymph was calculated by the following formula: $[HCO^{-3}] = [CO_2] - (\alpha\ CO_2 \cdot P_{CO_2})$. In addition to in vivo measurements, the P_{CO_2} at fixed hemolymph pH and bicarbonate values were calculated from the Henderson-Hasselbalch equation, in order to draw P_{CO_2} isopleths on the Davenport diagram presented in Figure 2. The constants pK_1 and αCO_2 were estimated at the appropriate temperature and ionic content using the nomograms prepared for *Carcinus* hemolymph by Truchot ('76) and used elsewhere for the terrestrial land crab *Coenobita clypeatus* (McMahon and Burggren, '79).

A second 200-μl sample was drawn within minutes of the sampling for hemolymph gases. The hemolymph was defibrinated by agitation followed by centrifugation, and the osmolality of this hemolymph sample was measured in an Advanced Instruments freezing point osmometer. This sample was recovered and then lyophilized in a 1.5-ml polyethyene centrifuge tube using a Unitrap II freeze dryer on board the Alpha Helix, and later analysed for ion and protein concentrations. Na^+, K^+, Ca^{++}, and Cu^{++} concentrations were determined with a Jarrell Ash 850 Atomic Absorption Spectrophotometer on samples diluted 1/100 with double-distilled water. Cl^- levels were measured using a chloride-sensitive electrode system (Orion 94-17A electorde, Beckman 4500 meter) calibrated with Orion Cl^- standards. Protein was measured using the BioRad technique on a Pye Unicam SP8-150 Spectrophotometer.

Oxygen equilibrium curves were determined on pooled samples of hemolymph (0.3 ml from each of at least four individuals, by a mixing technique similar to that of Lenfant and Johansen ('65) and described in detail for crustacean blood by McMahon and Burggren ('79). The pH of the hemolymph at P_{50} was adjusted by equilibrium with gas mixtures of appropriate P_{CO_2}.

Experimental protocol

All animals of each species were given an undisturbed acclimation period of 4–6 days under temperature and humidity conditions described above, and were given free access to food and water. At the end of this period, these

animals were assumed to be in a normal water and salt equilibrium, and were termed "normal hydrated" crabs. Hemolymph samples were then taken, treated, and analyzed as described above. All individuals were weighed daily during this and all subsequent periods. In the case of *Coenobita*, the animals were not extracted from their molluscan shells. Rather, the weight of the animal plus shell (plus shell water) was determined. Then, at the end of the experiments, the animal was removed from the shell, the shell weight was determined, and this value was then subtracted from the recorded total weight data.

After this acclimation period, all drinking dishes, food items, and leaf litter were removed from the cages, and dehydration was begun. Air temperature and relative humidity remained within the same range evident during equilibration. *Coenobita* were allowed to undergo desiccation within their molluscan shell and with whatever water was stored within. Similarly, *Cardisoma* and *Birgus* were allowed to retain whatever water existed in their branchial cavity or on other body surfaces at the start of dehydration.

After a severe dehydration period of 3–4 days (*Cardisoma, Birgus*) or up to 6–7 days (*Coenobita*), during which the animals were weighed daily, a second set of hemolymph samples was taken for analysis, these representing the "dehydrated" state. These dehydration periods caused substantial decreases in body weight and concomitant increases in hemolymph osmolality (see Results) although, importantly, most crabs remained mobile and were often aggressive. Preliminary experiments had revealed that a further 24 hours of dehydration for any of the three species would lead to a comatose state and, invariably, death within another few hours. "Dehydrated" crabs, as sampled for hemolymph, were thus suffering a critical water loss, and although a small proportion (10–20%) did not recover, the rest had the full potential for recovery.

After hemolymph sampling was completed for the dehydrated state, 1–2 cm of fresh water (or 50% seawater in the case of a separate group of six *Cardisoma,* plus a large amount of leaf litter providing a dry platform for emergence, as an alternative to soaking, was placed in the terraria. After approximately 24 hours in these conditions, these "rehydrated" animals were weighed and then sampled a third and final time for hemolymph.

The only deviation from this protocol was in the case of *Birgus*, in which the initial hydration hemolymph samples were not analyzed for ion content, and so dehydration values for these variables were contrasted with rehydration values.

Statistical treatment of data

All differences between mean values were tested for significance using the two-tailed Student's t-test for independent means, and a significance level of $P < 0.05$.

RESULTS

Normal values for body weight, hemolymph osmolality, and total body water distributed throughout the tissues, as well as values during dehydration and rehydration, are given in Table 1 for *Cardisoma, Birgus,* and *Coenobita.* Figure 1 summarizes the data on percentage changes in hemolymph osmolality and ion and protein levels during dehydration and rehydration. After measurement of osmolality, the samples were lyophilized, and then reconstituted to the original volume 2 months after the original sampling. Although great care had been taken to return the samples to their original volume, the total number of measured electrolytes exceeded the osmolality measured in that sample before lyophilization and reconstitution by approximately 10–30%. This suggested that at some stage in sample storage and handling after measurement of osmolality, evaporation from the sample had occurred. This presumably affected ion and protein concentrations, but not the percentage changes of the ions and hemolymph proteins compared to original hydrated levels. Figure 1 thus expresses electrolyte levels as a percentage of that measured in hemolymph from crabs in the normal hydrated state.

Changes in body water and hemolymph ions

Both the rate at which dehydration occurred, and the ability to withstand water loss, varied considerably between the three genera examined. *Cardisoma* and *Birgus*, which began dehydration with only the limited water stores within their branchial chambers, experienced a severe dehydration and significant rise in hemolymph osmolality in just 3–4 days. *Coenobita*, which started the dehydration period with an indeterminate store of water within its molluscan shell, reached a near-lethal dehydration only after 6–7 days, but suffered a larger loss of body weight and total water than either *Cardisoma* or *Birgus* (Table 1).

Hemolymph osmolalities in *Birgus* exceeded those in *Cardisoma* by 20–50%, at all hydration states, but similar percentage changes in ion ratios and protein levels occurred in both

TABLE 1. Effects of dehydration at 28–30°C on body weight, hemolymph, and tissue water content and hemolymph osmolality in three supralittoral crabs

	Cardisoma	*Birgus*	*Coenobita*
Number of animals	10	7	5
Hydrated body weight (g)	262 ± 70	44 ± 34	44 ± 13
Dehydrated body weight (g)	228 ± 64	38 ± 29	36 ± 11
Dehydrated weight loss (g)	34 ± 22	6 ± 6	8 ± 3
Body weight loss	13%	14%	18%
Body water loss	18%	21%	28%
Hydration hemolymph osmolality (mOsm/Kg H_2O)	649 ± 34	779 ± 25 (n = 2)	807 ± 23
Dehydration hemolymph osmolality (mOsm/Kg H_2O)	811 ± 140	1,013 ± 117	1,068 ± 85
Increase in osmolality (mOsm/Kg H_2O)	165 ± 50	240 ± 87	261 ± 37
Increase in hemolymph osmolality	25%	31%	32%

Mean values ± 1 standard deviation are given. Percentage values were calculated from mean values of body weight and osmolality for each species. Body weight is assumed 65% water for all three species (Harris and Kormanik, '81).

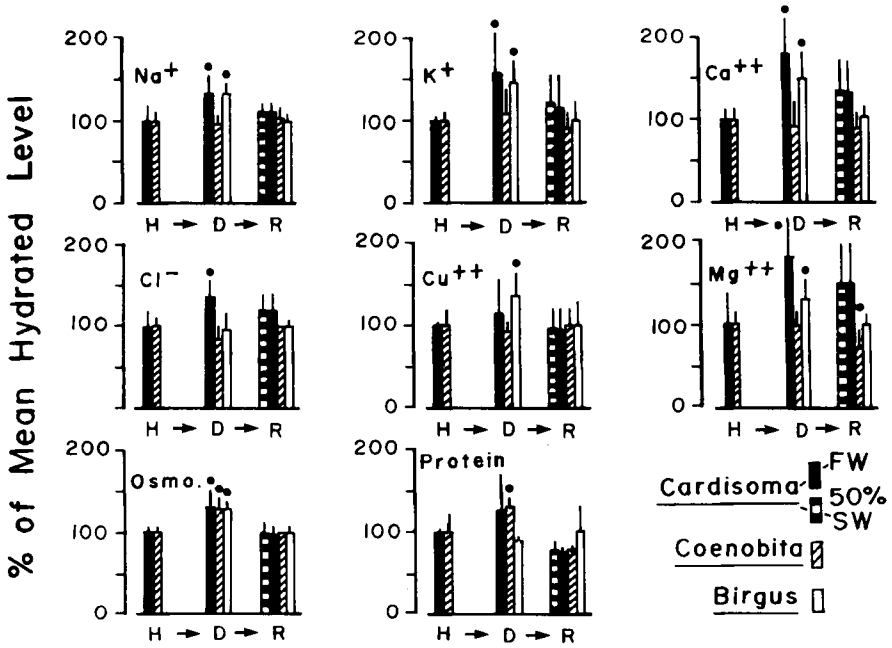

Fig. 1. Percentage changes in hemolymph ion and protein concentration and in hemolymph osmolality in *Cardisoma carnifex*, *Birgus latro*, and *Coenobita clypeatus* in the normal hydrated state, after severe dehydration, and following 24 hours of rehydration at 28–30°C. Mean values ± 1 standard deviation are given. Data for *Cardisoma* rehydrated in 50% seawater, rather than tap water, are also indicated. Numbers of *Coenobita* sampled during the normal hydrated state, dehydrated, and rehydrated are 5, 5, and 6, respectively. Numbers of *Birgus* sampled during dehydration and rehydration states are 7 and 8 respectively. Number of *Cardisoma* sampled during hydrated, dehydrated, rehydrated (tap water), and rehydrated (50% seawater) states are 5, 11, 6, and 6, respectively. A solid dot above the s.d. bar indicates a significant difference ($P < 0.05$) from either the original hydrated state (*Cardisoma, Coenobita*), or the rehydration state (*Birgus*). See text for experimental protocol.

crabs (Fig. 1). Almost all measured monovalent and divalent cations increased significantly during dehydration and the resulting significant rise in hemolymph osmolality. After 24 hours of rehydration in tap water, these variables had returned to values not significantly different ($P > 0.10$) from those measured in the original, hydrated state. In a group of six *Cardisoma*, rehydration in 50% seawater rather than tap water returned all ion levels and hemolymph osmolality to values not significantly different ($P > 0.10$) from those produced by tap water rehydration.

Coenobita showed quite different responses to body water loss. In spite of a significant increase ($P < 0.05$) in hemolymph osmolality during dehydration and a decrease during rehydration, no significant changes occurred in the ratios of any hemolymph ions.

Effects on oxygen transport

In the normal, hydrated state, the O_2 partial pressure of both prebranchial (venous) hemolymph (P_vO_2) and postbranchial (arterial) hemolymph (P_aO_2) was similar in all three crabs (Table 2–4). However, the O_2 half-saturation point, P_{50}, of the hemolymph under simulated in vivo conditions of pH and P_{CO_2} ranged from 11 mmHg in *Cardisoma* to 21 mmHg in *Birgus*. Use of the appropriate hemolymph O_2 equilibrium curve for each species (McMahon and Burggren, '81) indicates that in the normal, hydrated state postbranchial hemolymph was normally 65–80% O_2 saturated in all three species. Total hemolymph oxygen capacity as well as the O_2 content differences between pre- and postbranchial hemolymph varied considerably both between individuals and between species, with *Coenobita* having the highest capacity and the highest a–v O_2 content difference. (Higher values of P_aO_2 are reported for *Cardisoma* by Wood and Randall ('81). They maintained their crabs in a maximally hydrated state, obfuscating direct comparison of data (see McMahon and Burggren, '81).)

Cardisoma and *Birgus* showed similar respiratory responses to dehydration, with little evidence of any substantial breakdown in oxygen exchange or transport. Under in vivo conditions the hemolymph of critically dehydrated individuals retained essentially the same O_2 carrying characteristics during dehydration as in the normal hydrated state, with comparatively small increases in in vivo P_{50} occurring. Both *Cardisoma* and *Birgus* showed significant increases in P_aO_2 when compared to their normal hydrated state, and in *Birgus* this resulted in a significant elevation in postbranchial O_2 content (Table 3). The O_2 content difference between pre- and postbranchial hemolymph rose slightly but not significantly during dehydration for both *Cardisoma* and *Birgus*.

Coenobita, however, suffered major disruption to hemolymph oxygenation after dehydration. P_aO_2 fell by one-half, resulting in only a 30% O_2 saturation of postbranchial hemolymph (Table 4). Mean hemolymph O_2 capacity actually decreased during the dehydration period, and a significant ($P < 0.05$) fall in postbranchial O_2 content and prebranchial O_2 content difference developed.

The extent and speed of recovery of values related to O_2 transport following rehydration also varied between species (Tables 2–4). While *Birgus* achieved a nearly complete recovery of hemolymph O_2 values upon rehydration, in *Coenobita* the P_{O_2} of both post- and prebranchial hemolymph and prebranchial O_2 content remained depressed from normal hydration values. In *Cardisoma*, in spite of a return toward rehydration values of P_aO_2, C_aO_2 was significantly depressed upon rehydration. Delays in recovery of O_2 transport were not the result of changes in the in vivo O_2 equilibrium curve in any of the species tested.

Carbon dioxide and acid-base balance

Mean values of P_aO_2 and pH_a measured in hydrated crabs were essentially similar in all three species (Tables 2–4, Fig. 2). Lower P_{CO_2} and higher pH values tended to occur in *Birgus*, whereas the converse tendency was seen in *Cardisoma*. Mean prebranchial P_{CO_2} levels and pH values were generally higher and lower, respectively, than their postbranchial counterparts, but interanimal variability was often sufficiently great to prevent demonstration of a significant difference. Carbon dioxide content of prebranchial hemolymph in normal, hydrated *Coenobita* and *Cardisoma* was considerably higher than that in *Birgus*. HCO_3^- concentration calculated from mean values of pH and P_{CO_2} for all three species was 85–99% of the measured total CO_2 of the hemolymph.

The major effect of dehydration on acid-base balance in all three species was a fall in the pH of post- and prebranchial hemolymph (Tables 2–4, Fig. 2). The measured P_{CO_2} of venous hemolymph either showed no change (*Cardi-*

TABLE 2. Effects of dehydration and rehydration at 28–30°C on gas transport and acid-base balance of Cardisoma carnifex

	Hydration	Dehydration	Rehydration
Number of animals	5	10	6
Body wt. (g)	291 ± 78	261 ± 76*	293 ± 82
Hemolymph osmolality (mOsm/Kg H_2O)	649 ± 34 (10)	811 ± 140(7)*	632 ± 66
P_aO_2 (mmHg)	23 ± 8	37 ± 7*	30 ± 9
P_vO_2 (mmHg)	12 ± 1	9 ± 5	13 ± 8
P_{50}, in vivo conditions	11	—	11
P_{50}, pH 7.46	13	—	14
C_aO_2 (mM/l)	0.81 ± 0.27	0.77 ± 0.38	0.50 ± 0.15*
C_{vO2} (mM/l)	0.38 ± 0.15(4)	0.27 ± 0.23	0.23 ± 0.15
a–v O_2 diff. (mM/l)	0.42 ± 0.27	0.50 ± 0.35	0.27 ± 0.23
$C_{O2\ max}$ (mM/l)	1.08	1.42	0.77
pH_a	7.58 ± 0.10	7.47 ± 0.09*	7.59 (1)
pH_v	7.61 ± 0.10	7.44 ± 0.11	7.56 ± 0.06
P_aCO_2 (mmHg)	11 ± 2	12 ± 6 (5)	9 ± 2 (3)
$P_v\ CO_2$ (mmHg)	12 ± 1	14 ± 3	12 ± 3
C_aCO_2 (mM/l)	19 ± 2	17 ± 7	—
C_vCO_2 (mM/l)	19 ± 2	17 ± 6	16 ± 6
$[HCO_3]_v$ (mM/l)	18.6	16.5	15.6

Mean values ± 1 standard deviation are given. Number of individuals used, when different from that indicated in the first row, are given in parentheses. Asterics indicate a significant ($P < 0.05$) difference when compared to original hydration values, using an independent t-test (a dependent t-test for body weight, only). Hemocyanin P_{50} values and hemolymph oxygen capacity (C_{O2} max) were determined on a pooled hemolymph sample. Bicarbonate concentration of venous hemolymph [$HC0_3^-$] was calculated from mean values of hemolymph pH and P_{CO2}. See text for further details.

TABLE 3. Effects of dehydration and rehydration at 28–30°C on gas transport and acid-base balance of Birgus latro

	Hydration	Dehydration	Rehydration
Number of animals	9	7	7
Body wt. (g)	89 ± 72	78 ± 64	87 ± 72
Hemolymph osmolarity (mOsm/kg H_2O)	779 ± 25 (2)	1013 ± 117*	775 ± 63
P_aO_2 (mmHg)	—	51 ± 17 (5)	27 ± 5
P_{vO2} (mmHg)	13 ± 4	16 ± 4	12 ± 2
P_{50}, in vivo conditions	21	23	19
P_{50}, pH 7.46	25	22	24
C_aO_2 (mM/l)	—	1.08 ± 0.19(5)*	0.73 ± 0.23
C_{vO2} (mM/l)	0.38 ± 0.27	0.42 ± 0.31	0.19 ± 0.08
a–v O_2 diff. (mM/l)	—	0.65 ± 0.19(4)	0.42 ± 0.23(5)
$C_{O2\ max}$ (mM/l)	0.89	1.23	1.0
pH_a	—	7.63 ± 0.07(5)	7.70 ± 0.05
pH_v	7.61 ± 0.11	7.55 ± 0.10	7.64 ± 0.06
P_{aCO2} (mmHg)	—	7 ± 1 (5)*	9 ± 1
P_vCO_2 (mmHg)	7 ± 1	6 ± 1	8 ± 2
C_aCO_2 (mM/l)	—	—	—
C_vCO_2 (mM/l)	13 ± 3	9 ± 2	13 ± 3
$[HCO_3]_v$ (mM/l)	12.5	8.8	12.7

See legend of Table 2 for explanation of symbols.

TABLE 4. Effects of dehydration and rehydration at 28–30°C on gas transport and acid-base balance of Coenobita brevimanus

	Hydration	Dehydration	Rehydration
Number of animals	9	5	8
Body wt. (g)	46 ± 13	40 ± 12*	44 ± 13
Hemolymph osmolarity (mOsm/Kg H_2O)	807 ± 23(5)	1068 ± 85*	650 ± 30*
P_aO_2 (mmHg)	24 ± 8(8)	12 ± 2(4)	19 ± 4(7)
P_vO_2 (mmHg)	14 ± 2(7)	10 ± 2*	11 ± 1(7)*
P_{50}, in vivo conditions	15	16	15
P_{50}, pH 7.46	17	14	16
C_aO_2 (mM/l)	1.08 ± 0.35(7)	0.62 ± 0.12(4)*	0.73 ± 0.27(7)
C_{vO_2} (mM/l)	0.31 ± 0.15	0.31 ± 0.15	0.19 ± 0.12(7)
A–v O_2 diff. (mM/l)	0.73 ± 0.42	0.31 ± 0.23	0.54 ± 0.27(6)
$C_{O_2\ max}$ (mM/l)	1.58	1.42	1.19
pH_a	7.68 ± 0.13(8)	7.44 ± 0.19*	7.64 ± 0.08(6)
pH_v	7.57 ± 0.05(8)	7.37 ± 0.10(8)*	7.54 ± 0.11
P_{aCO_2} (mmHg)	9 ± 3	9 ± 2	8 ± 2
P_{vCO_2} (mmHg)	14 ± 2	10 ± 3*	9 ± 2*
C_aCO_2 (mM/l)	23 (4)	10(4)*	—
C_vCO_2 (mM/l)	19 ± 3	12 ± 4*	16 ± 3*
$[HCO_3]_v$ (mM/l)	18.5	11.7	15.7

See legend of Table 2 for explanation of symbols.

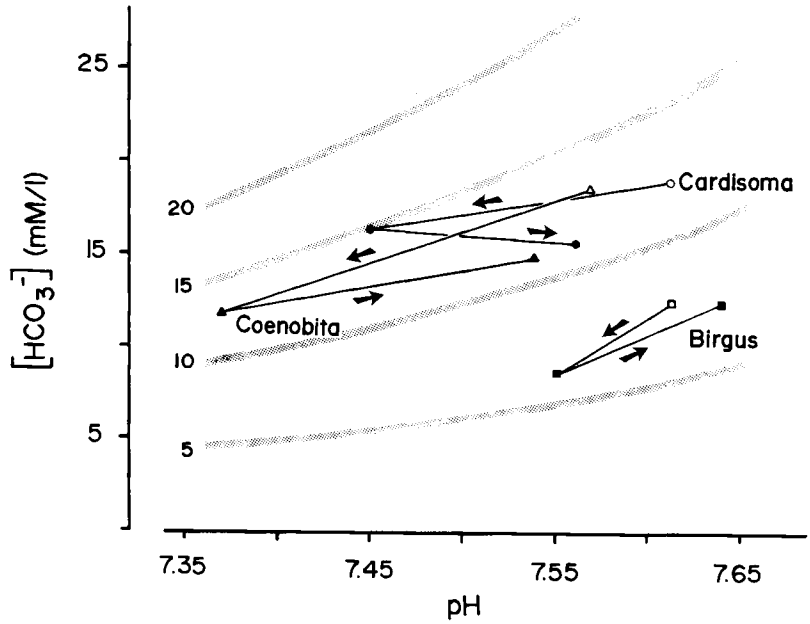

Fig. 2. A Davenport diagram indicating changes in acid-base status in the prebranchial hemolymph of *Cardisoma*, *Birgus*, and *Coenobita*, beginning with the normal hydrated state (open symbols), proceeding to severe dehydration (arrows), and following 24 hours of rehydration. Calculated P_{CO_2} isopleths are indicated by the shaded lines. P_{CO_2} units are in mmHg.

soma) or decreased slightly (*Coenobita, Birgus*). HCO_3^- levels fell sharply, particularly in *Coenobita* and *Birgus*.

Within 24 hours following return to "rehydration" conditions, hemolymph pH had returned to hydrated levels in all three species. In *Birgus* and to a lesser extent *Cardisoma*, P_{CO_2} levels were also returned to near-hydration values. Bicarbonate concentrations also returned to predessication levels in *Birgus*, but not in *Cardisoma* or *Coenobita*. Here, as above, *Coenobita* again proved exceptional as neither P_{CO_2} nor bicarbonate levels had returned to hydration levels following 24 hours of rehydration.

DISCUSSION

Dehydration and hydromineral regulation

Cardisoma and *Birgus* both reached critical dehydration well before *Coenobita*. In fact, both de Wilde ('73) and McMahon and Burggren ('79) reported that *Coenobita clypeatus* could survive 7–12 days under similar conditins, 2–3 times longer than we observed for the other two genera. Strict comparison between rates of body water loss is problematic because of the undetermined amount and use of water contained within *Coenobita's* shell and within the branchial chambers of *Birgus* and *Cardisoma*. Pinder and McMahon (unpublished) observed that shell water was definitely drawn upon to offset water loss from body tissues during 3-day periods of dehydration in *Coenobita clypeatus*. In addition, the shell of *Coenobita* may provide a humid microenvironment that would slow the rate of diffusional water loss from the abdomen and other moist surfaces (McMahon and Burggren, '79).

The relationships between loss of body water and changes in hemolymph osmolality and ion levels in these three diverse terrestrial crabs are very complex. In *Cardisoma* and *Birgus*, substantial increases in hemolymph ion levels occur during dehydration, yet the percentage increases in Ca^{++}, K^+, and Mg^{++}, for example, are greater than in Na^+ and Cl^-. Disproportionate changes in ion concentrations during dehydration have also been reported for several terrestrial brachyurans (Gross, '63). Such changes cannot reflect a simple loss of water from the hemolymph during dehydration, and probably indicate some form of active regulation of these ions.

During dehydration, *Coenobita*, unlike most other terrestrial crabs, exhibited only very

small changes in hemolymph ion levels, even though total body water decreased by nearly one-third. An active redistribution of osmotically important ions, and hence of water, between the nonvascular compartments and the hemolymph may have occurred during dehydration. Alternatively, shell water could serve as a "dump" for inorganic ions as their tissue and blood levels begin to rise (de Wilde, '73; Pinder and McMahon, unpublished). The gills of *Coenobita* can be in intimate contact with the shell water, and may well serve an active role in ion exchange, as gills do in many other Crustacea (McDonald et al., '79; Cameron, '78a,b; Cameron and Batterton, '78).

The restoration of body water and ion levels in all three genera was nearly complete within 24 hours. In *Cardisoma*, interestingly, rehydration in either tap water or 50% seawater made little difference to the restoration of hemolymph ion levels and osmolality. The salinity of the water in the burrows of this species is highly variable (R. Harris, personal communication). This brachyuran crab is apparently well able to maintain a preferred blood composition in the face of a widely varying ionic environment, as can *Coenobita* (de Wilde, '73).

Oxygen transport at critical dehydration

Increases in hemolymph concentration of inorganic ions, particularly Ca^{++} and Mg^{++}, generally induce a leftward shift of the O_2 equilibrium curve for hemocyanin (Larimer and Riggs, '64; Spoek, '67; Weiland and Mangum, '75; Truchot, '75; reviewed for crustaceans by Mangum, '81). The substantial increases in Ca^{++} and Mg^{++} levels in *Birgus* and *Cardisoma* could have accounted for the in vitro fall in the P_{50} (Tables 2–4), especially since much smaller changes influence O_2 affinity in crustacean hemocyanins (Truchot, '75). However, a decrease in P_{50} developed in *Coenobita*, even though the levels of Ca^{++} and Mg^{++} remained virtually unchanged during dehydration and rehydration, so other ions or organic substances that increase hemocyanin–O_2 affinity must be implicated in this crab.

The hemocyanins of all three crabs exhibited a significant Bohr shift (McMahon and Burggren, '81), and the decrease in pH during dehydration would, if it occurred in isolation, induce a rightward shift in the O_2 equilibrium curve of about 3–4 mmHg. This effect is apparently countered by the tendency for a leftward shift through increases in inorganic ions or other substances, so that only very small net changes in O_2 affinity result from changes in hydration state. Salinity and pH effects on hemocyanin–O_2 affinity interact similarly in the euryhaline crab *Callincetes sapidus*, so that an "enantiostasis" with respect to O_2 transport by hemocyanin occurs (Weiland and Mangum, '75). It is interesting that these opposing processes apparently can also be adaptive for a respiratory homeostasis during dehydration in terrestrial decapods.

The substantial rise in P_aO_2 in *Cardisoma* and *Birgus* during dehydration could have resulted from either an increase in the transit time of hemolymph through the respiratory surface (i.e., a reduced rate of hemolymph flow) or an increase in the flow of air through the branchial chambers. The latter could be maladaptive, if there is a large loss of water from the respiratory membranes. An increase in hemolymph transit time, on the other hand, could have resulted from an increase in hemolymph viscosity and the resultant decrease in hemolymph flow during dehydration. However, the hemolymph O_2 capacity rose (probably a simple result of hemoconcentration) and 'a–v O_2 content differences were maintained or even increased, so mass transport of O_2 to the tissues could thus have been maintained.

Coenobita was again distinctive, in that during dehydration hemolymph O_2 capacity decreased, and a marked failure to oxygenate postbranchial hemolymph was observed (Table 4). *Coenobita* experienced the greatest decrease in serum pH during dehydration, and some dissociation of hemocyanin into distinct subunits with a collectively lowered O_2 capacity is possible. Hemolymph ion and protein concentration increased by nearly 50%, whereas total hemolymph Cu^{++} did not change.

In *Coenobita,* which suffered the greatest disruption to hemolymph oxygenation during dehydration, 24 hours of rehydration was insufficient to restore the transport processes fully, even though blood osmolality and tissue water were restored to control levels. Considering the comparatively small adjustments in oxygen transport occurring during dehydration in *Cardisoma* and *Birgus*, it is not surprising that rehydration served rapidly to restore most features of hemolymph oxygenation to near-hydration in these two genera. Hemolymph oxygenation in *Cardisoma* and *Birgus* thus appears to be largely independent of all but the most severe changes in body water and salts.

Acid-base balance during dehydration

Plasma pH varies inversely with blood salinity in several invertebrates, including brachyuran crabs (Fynh et al, '72; Laird, '73; Truchot, '73; Weiland and Mangum, '75). These adjustments in pH result not from changes in hemolymph CO_2, but rather from changes in plasma NH_3 or other unidentified bases derived from cellular metabolism (Truchot, '75; Weiland and Mangum, '75). The present study indicates some parallels between acid-base regulation in response to dehydration. In *Birgus* and *Coenobita* and to a lesser extent in *Cardisoma*, the acidosis resulting from dehydration and the resulting ionic changes is apparently largely metabolic in origin, since no significant increase in P_{CO_2} occurred (Fig. 2). The source of the acidosis is not known, but in each case loss of bicarbonate occurred. The loss of bicarbonate actually exceeds that shown in Tables 2–4 since these losses occurred in the face of a 25–32% hemoconcentration (Table 1). Reduction of HCO_3^- might be beneficial during dehydration in reducing the osmolality increase associated with water loss. In at least *Cardisoma* and *Coenobita*, where sizable water stores occur in or near the branchial cavity, it is possible that excretion of bicarbonate may occur directly across the gills at least during the early dehydration stages. Pinder and McMahon (unpublished data) have shown that bicarbonate builds up in shell water of *Coenobita clypeatus* between visits to water, but the mode of excretion has not been established.

Loss of bicarbonate could also occur indirectly via CO_2 efflux across the respiratory surfaces. Randall and Wood ('81) and Wood and Randall ('81) discuss CO_2 excretion in hydrated *Cardisoma*, and demonstrate a considerable ability for increased CO_2 efflux following activity. Presuming that a similar potential occurs in the other two species (similarly large P_{CO_2} gradients exist across their gills), a classic respiratory compensation for the dehydration acidosis could theoretically occur, but does not. An increased ventilation for a respiratory compensation of metabolic acidosis could hasten dehydration through evaporative water loss from the respiratory surfaces, and may not be an appropriate response when body water is decreasing.

The exoskeleton of decapod crustaceans represents a considerable store of carbonate, which can be liberated as a buffer in marine crabs (Truchot, '75; deFur et al., '81) and in *Cardisoma* (Wood and Randall, '81) to allow compensation for metabolic acidosis. The disproportionately large increase in Ca^{++} ion during dehydration (Fig. 1) suggests that this buffering source could also be involved in this circumstance. Following rehydration, bicarbonate levels are restricted in *Birgus* within 24 hours but longer periods are involved for both *Coenobita* and *Cardisoma*. Considering the ready availability of metabolic CO_2, these delays are difficult to explain. Nonetheless, when marked loss of CO_2 reserves accompanied activity in *Cancer magister* (McDonald et al., '79), recovery also took from 24 to 48 hours. Movements of HCO_3^- across the gills are correlated with the movement of other ions, especially Cl^-, and thus the delayed restoration may be associated with some other ions and regulatory process. Alternatively, if erosion of shell carbonates had occurred during the dehydration process, then hemolymph HCO_3^- levels may be lowered while these shell carbonates stores are replenished.

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