

APPARENT  $H^+$  EXCRETION AND  $CO_2$  DYNAMICS  
ACCOMPANYING CARAPACE MINERALIZATION IN THE  
BLUE CRAB (*CALLINECTES SAPIDUS*) FOLLOWING  
MOULTING

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SUMMARY

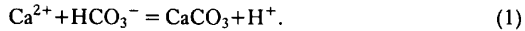
Just after moulting the blue crab, *Callinectes sapidus*, is soft and defenceless, but in the following days, a hard calcareous shell is formed for support and protection. The carapace of intermoult (hard) crabs constitutes 27% of the wet weight and 53% of the dry weight, with 14% of the body water contained in a carapace fluid compartment. The pH of the shell fluid compartment is maintained at 0.3 to 0.5 pH units above that of the blood. The mineral portion of the carapace is primarily  $CaCO_3$ , with minor components of  $Mg^{2+}$  and  $PO_4^{-3}$  plus traces of other ions. The formation of a new shell after moulting is accomplished by apparent  $H^+$  excretion and  $Ca^{2+}$  uptake at remarkable rates, averaging 12.5 and reaching as high as 21 mequiv  $kg^{-1} h^{-1}$ . Direct bicarbonate uptake from the sea water appears to be an important component of the apparent  $H^+$  efflux, since  $CO_2$  is deposited in  $CaCO_3$  at a rate greater than metabolic production, and the animals show a net  $CO_2$  uptake for several days.

INTRODUCTION

A distinctive feature of the arthropods' exoskeletal support system is that in order to increase in size, the entire structure must be shed and a new one periodically built. In the crabs the soft organic part of the new carapace is partly formed beneath the old one before moulting (Passano, 1960), but the mineral salts that lend it strength must be deposited after moulting, when the new carapace has expanded to its new size (Robertson, 1937, 1960; Greenaway, 1974, 1983; Welinder, 1975). This must be done quickly, as the animal is extremely vulnerable to predators when soft. The principal mineral components of the carapace are  $Ca^{2+}$ ,  $Mg^{2+}$ , carbonate and phosphate, with  $CaCO_3$  as the predominant salt (Clarke &

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Wheeler, 1922; Welinder, 1974). The new  $\text{CaCO}_3$  is formed according to the overall reaction:



Thus a high rate of carbonate deposition implies both a high rate of  $\text{Ca}^{2+}$  transport, and an equal net  $\text{H}^+$  efflux from the deposition site. Little is known of the transport mechanisms involved in this process, either for crabs or molluscs (Campbell & Boyan, 1974; Roer, 1980), although it is known that freshwater and terrestrial crustaceans often conserve the  $\text{Ca}^{2+}$  from the old carapace by transporting it to gastroliths, which are retained during the moult, and used for the new carapace (Passano, 1960). Most marine animals apparently do not form gastroliths, which is probably due to the relatively high availability of  $\text{Ca}^{2+}$  in sea water.

The new carapace in the blue crab, *Callinectes sapidus*, hardens quickly after moulting, reaching the 'paper shell' stage in only 2 or 3 h, and feels as hard as the intermoult crab after about 2 days. Whereas the mineralization process has been studied in some detail, both in *Callinectes sapidus* and other crustaceans (Drach, 1937; Kleinholz, 1941; Vigh & Dendinger, 1982), the questions of acid-base consequences and  $\text{CO}_2$  dynamics have not been studied. The objectives of this study were to determine the relationships between the mineralization process,  $\text{CO}_2$  balance and overall acid-base balance.

#### MATERIALS AND METHODS

Blue crabs (*Callinectes sapidus* Rathbun), weighing between 10 and 350 g, were either purchased from local crabbers on the day of capture, or were collected by various means in the vicinity of Port Aransas, Texas. They were maintained in running sea water at temperatures between 20 and 27 °C. At the time of capture, the moult stage was assessed by examination of the various moult 'signs' (Perry, Ogle & Nicholson, 1979), and those judged to be in the immediate pre-moult stages were isolated in smaller aquaria. Intermoult crabs were fed periodically with fish, but the pre-moult crabs usually did not eat, nor were they given food after moulting. Some individuals were brought into the laboratory in the 'softshell' stage (within 2 h after moult), although the survival of these animals was much lower than that of crabs that moulted in the laboratory.

Wet weights of the crabs were obtained after allowing the water to run out of the gill chamber, and blotting the animals gently to remove excess external water. Dry weights of the whole animals were obtained by drying in an oven at 65–70 °C to constant weight. To assess the wet weight of the carapace, the whole animal was carefully dissected, the muscle tissue removed from internal 'struts' as well as the external portions, and the carapace pieces weighed, making sure they were kept moist until weighing to avoid evaporative losses. The dissected carapace pieces were then dried under the same conditions to assess the total dry weight of the carapace.

After drying, the carapace components or, in some cases, pieces of carapace removed from the animals, were extracted in 2 N-HCl for several days for analyses of the mineral (or acid-extractable) materials. The non-extractable material remaining was rinsed in de-ionized water, dried and re-weighed, and the weight of

acid-extractable material obtained by the difference in dry weight before and after acid extraction. The total volume of acid used in extraction was measured, and aliquots saved for various analyses. Some shed carapaces were treated similarly, i.e. blotted of excess water, weighed and carried through the same drying and extraction process.

Beginning immediately after completion of the moult, or as soon as animals were brought into the laboratory in the soft condition, they were placed in aquaria of known volume, with a ratio of sea water to crab of at least 400 to 1. Initial samples of sea water were taken, and thereafter at intervals up to 7 days, with changes of water as appropriate (see below). For each set of sea water samples, the ammonia content was measured by the phenolphthalein method (Solorzano, 1969), and the titratable alkalinity measured by titration of a 10 ml aliquot to pH 4.00 with 0.020 N-HCl. The difference between successive titration values (reduction in titratable alkalinity) was expressed as an 'apparent  $H^+$  excretion' after adding the contribution of  $H^+$  ions bound as  $NH_4^+$ . 'Apparent  $H^+$  excretion' is an operational term; 'apparent base uptake' or 'excretion of acidic equivalents' would be equally valid, since the effect on the external solution is in all cases the same, and the actual ion species moving are not known. The acidification of the sea water just after moulting was rapid, and the sea water was changed before 30% of the titratable alkalinity was used up.

At measured times following the moult, the crabs were killed, weighed, dried and weighed again. Some carcasses were cut into small pieces and extracted for several days in 2 N-HCl for assessment of the whole body titratable base and ion content.

The pH of the fluid compartment contained within the carapace was measured using the  $^{14}C$ -DMO (5,5-dimethyl-2,4-oxazolinedione) method with a simultaneous  $^3H$ -inulin marker for trapped extracellular fluid (Waddell & Bates, 1969; Cameron & Kormanik, 1982). These markers were allowed to equilibrate for at least 12 h before terminal sampling (see Wood & Cameron, 1985 for details). Samples of blood and carapace were combusted, along with appropriate standards, in a sample oxidizer (Packard C306) and counted in a liquid scintillation counter (Packard 3255), using both internal and external standards for efficiency correction. The blood pH was measured on samples withdrawn by syringe from the pericardial sinus, and the 'intracellular' pH calculated with the formulae given by Cameron & Kormanik (1982). 'Intracellular' may not be an appropriate term for the carapace fluid compartment; it is probably made up in part by the intracellular fluids of the epithelial cells, and in part by secreted extracellular fluids, but it behaves like an intracellular pool in excluding inulin ( $M_r$  approximately 5000) and admitting DMO. The underlying epithelial tissue was removed as completely as possible from all carapace samples by scraping and blotting with tissues.

In another series of experiments, post-moult crabs were placed gently into a small blackened respirometer supplied with well-aerated sea water. The flow was adjusted so that the difference between inflow and outflow  $O_2$  tension was about 30–40 Torr, which allowed measurement of  $O_2$  consumption using the water flow rate, the inflow-outflow difference and the solubility coefficient of sea water at the test temperature. Simultaneous paired samples of inflow and outflow were also analysed for total  $CO_2$  content with a conductometric apparatus (Capni-Con II, Cameron

Instrument Co.) to allow similar calculation of the net  $\text{CO}_2$  output. Since this difference was near the limits of accuracy of the method, 10 to 20 pairs of samples were analysed for each day post-moult, and the averages used. The respirometer was supplied from an 8-litre reservoir, to which the water was returned and vigorously re-aerated. Samples were taken from the reservoir for the measurements of apparent  $\text{H}^+$  excretion and ammonia excretion, as described above. The temperature for both the respirometry and the flux measurements was kept between 20 and 22 °C.

The effects of the carbonic anhydrase inhibitor acetazolamide (AZ) on apparent  $\text{H}^+$  flux and calcification were assessed in six crabs at various times after moulting. The acetazolamide was solubilized by addition of NaOH to saline medium, then titrated with HCl back to a pH between 8.6 and 8.8 prior to injection. The dose given was calculated for each crab to produce a concentration of  $10^{-4} \text{ mol l}^{-1}$  of total body water, injected in a volume of  $10 \mu\text{l l}^{-1}$  body water. Following injection, flux measurements were made as described above for 24–48 h, after which the animal was killed for various analyses.

Ion analyses of the carapace and carcass extracts were carried out by standard chemical methods:  $\text{Na}^+$  and  $\text{K}^+$  by flame photometry,  $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$  and  $\text{Sr}^{2+}$  by atomic absorption, phosphate by a colourimetric test (Sigma). The carbonate content of the samples was calculated indirectly: first a sample of the extraction acid was back-titrated to pH 7 with standardized NaOH. Then aliquots of the acid extracts were similarly back-titrated, and the difference between the acid blank and the extracts was used to calculate the titratable base of the samples. We then determined by test titrations that the phosphate was two-thirds titrated by this procedure (i.e. from  $\text{H}_3\text{PO}_4$  at low pH to  $\text{HPO}_4^{2-}$  at pH 7), and subtracted two-thirds of the measured phosphate content from the total titratable base to obtain the carbonate by difference (cf. Table 1). All results are presented as mean  $\pm$  s.e., unless otherwise stated.

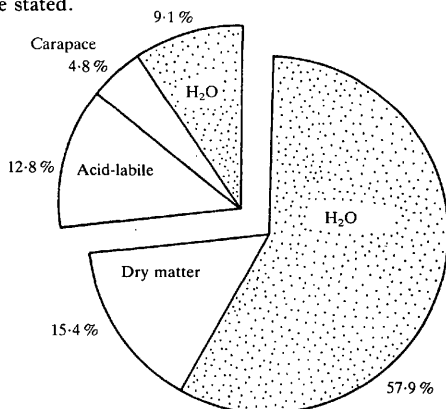


Fig. 1. The distribution of wet, dry and acid-labile weight of the exoskeleton (=carapace) as fractions of the total live (wet) weight of the animal on a 1 kg basis. The stippled portion represents water compartments and the unshaded portions the various dry matter components.

Table 1. *The composition of the mineral (acid extractable) portion of the carapace from intermoult blue crabs*

Species	g (%)	Equivalents
Ca <sup>2+</sup>	29.1 ± 0.5	1.45 ± 0.03
Mg <sup>2+</sup>	1.7 ± 0.05	0.14 ± 0.004
Sr <sup>2+</sup>	0.41 ± 0.03	0.01 ± 0.001
PO <sub>4</sub> <sup>3-</sup>	3.4 ± 0.1	0.11 ± 0.004
CO <sub>3</sub> <sup>2-</sup>	48.9	1.63
Titratable base		1.70 ± 0.06
Na <sup>+</sup>	1.3 ± 0.1	0.06 ± 0.004
K <sup>+</sup>	0.3 ± 0.04	0.01 ± 0.001
Total	85.1	

The data were obtained from 22 crabs and a total of 114 samples.

Means ± s.e. are given per 100 g of acid weight loss.

The carbonate concentration was calculated as the titratable base minus two-thirds of the phosphate concentration.

## RESULTS

### *The intermoult carapace*

The distribution of wet and dry weight for the whole dissected carapaces of seven intermoult crabs is shown in Fig. 1. The total body water content measured at 20°C was  $670 \pm 8 \text{ g kg}^{-1}$ , of which  $91 \text{ g kg}^{-1}$  (14%) was contained in the carapace. Of the dry weight of the whole carapaces a mean of  $72.8 \pm 1.4\%$  was extractable in acid. Samples of the carapaces taken from the mid-dorsal region and the ventral region beneath the branchial chambers (where the carapace is considerably thinner) averaged  $86.6 \pm 2.0\%$  ( $N = 30$ ) weight loss after acid extraction, reflecting the greater mineralization of these regions compared with the walking legs, internal 'struts' and other parts less important for protection. There was also a difference between the total water content of these pieces and the whole carapaces; the former had an average water content of  $26.4 \pm 1.8\%$  ( $N = 114$ ), the latter  $34.1 \pm 2.1\%$  ( $N = 7$ ).

The chemical composition of the intermoult carapaces is given in Table 1. The various materials analysed accounted for only 85.1% of the acid-extractable material. The balance would be partly Cl<sup>-</sup>, which could not be analysed since the extraction acid was HCl. The rest of the unaccounted-for material is probably made up of acid- and water-soluble proteins and water of hydration in the mineral matrix that was freed upon acidification (Vigh & Dendinger, 1982).

The 'intracellular' pH values for the carapace fluid compartment at 20°C averaged  $8.229 \pm 0.038$  ( $N = 17$ ), when the blood pH was  $7.852 \pm 0.014$  ( $N = 11$ ), a difference of 0.377 units. At 30°C, the carapace fluid compartment had a mean pH of  $8.123 \pm 0.035$  ( $N = 14$ ) and the blood of  $7.712 \pm 0.017$  ( $N = 14$ ), a difference of 0.411 units. The temperature slope of the blood was  $-0.014^\circ\text{C}^{-1}$ , and for the carapace  $-0.010^\circ\text{C}^{-1}$  (see Wood & Cameron, 1985).

*Composition of the shed carapace*

The percentage of water in mid-dorsal and branchial chamber samples of the shed carapaces (six crabs, 30 samples) analysed was slightly less than the intermoult carapaces,  $23.0 \pm 0.85$  compared to the value given above for samples from the same sites,  $26.4 \pm 1.8\%$  ( $0.10 > P > 0.05$ ). The chemical composition was not significantly different from the values given in Table 1 and Fig. 1 for intermoult crabs, nor was there an appreciable change in the total shell weight as a percentage of body weight, indicating little resorption of mineral material prior to moulting. The weight per unit area of samples of shed carapaces averaged  $0.114 \pm 0.012 \text{ g cm}^{-2}$  compared to  $0.103 \pm 0.003 \text{ g cm}^{-2}$  for intermoult crabs, but the residue after acid extraction was only  $9.1 \pm 0.9\%$  of the dry weight in the shed carapace samples, compared to  $18.0 \pm 1.4\%$  for intermoult crabs. The appearance of the dried residue was also quite different, all of which indicates that there was a significant resorption of organic material from the carapace before moulting, but no significant resorption of the mineral material.

*Carapace composition following moulting*

In the 7 days following moulting, the dry weight of carapace samples was at first quite low, and the percentage water content was higher than during intermoult (Fig. 2). Dry weight and water content gradually approached the intermoult values; the

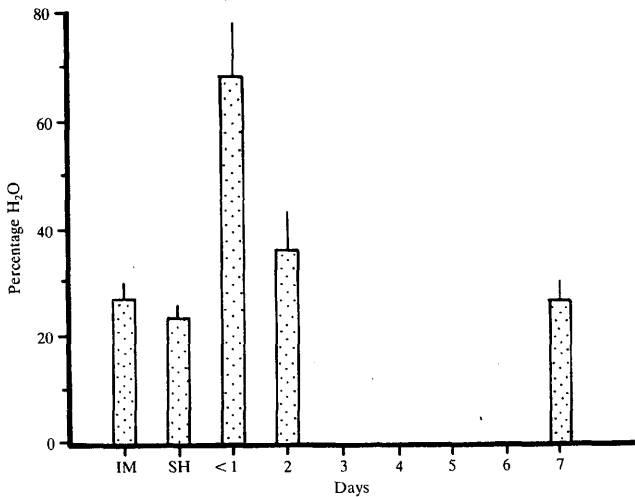


Fig. 2. The percentage water content of samples of dorsal and branchial chamber carapace from intermoult (IM) crabs, shed carapaces (SH) and newly moulted crabs at intervals up to 7 days following the moult. Vertical bars are  $\pm 1$  s.e.

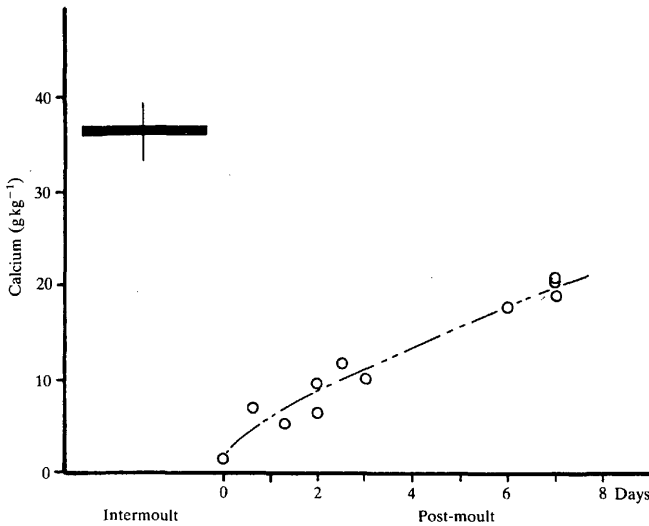


Fig. 3. The total body calcium content of intermoult crabs (solid bar) and newly moulted crabs at various times after the moult (open circles).

water content was not significantly different at 7 days post-moult, but the dry weight was only about half the intermoult value. The whole body calcium content was a good indicator of the progress of mineralization (cf. Table 1). The calcium content dropped precipitously just after moult, then rose at a rapid rate during the next 7 days (Fig. 3), reaching about 55% of the intermoult value. At the end of 1 week, an average of  $20.0 \text{ gm kg}^{-1} \text{ Ca}^{2+}$  had been deposited.

#### *Acid-base and CO<sub>2</sub> aspects of carapace mineralization*

Along with the deposition of calcium, there must be an equivalent net  $\text{H}^+$  movement out of the animal, and an equivalent supply of  $\text{CO}_2$  in some form. Measurements of apparent  $\text{H}^+$  fluxes from nine crabs during the first 7 days post-moult showed that the maximum apparent flux of about  $12.5 \text{ mequiv kg}^{-1} \text{ h}^{-1}$  was reached at about 1 day post-moult, typically after a few hours lag immediately after the moult (Fig. 4). In one individual, the peak rate recorded was  $21.5 \text{ mequiv kg}^{-1} \text{ h}^{-1}$ . The integrated area under the curve shown in Fig. 4 gives a measure of the total flux for the first 7 days, which averaged  $1.10 \text{ equiv kg}^{-1}$ , matching well with the  $1.0 \text{ equiv kg}^{-1}$  of  $\text{Ca}^{2+}$  accumulated (Fig. 3). Less than 1% of the net apparent  $\text{H}^+$  flux could be accounted for by ammonia excretion. An additional confirmation of the mineralization rate was obtained by comparing the observed total apparent  $\text{H}^+$  flux with the total titratable base of the post-moult carcasses. For six crabs killed between 3 and 7 days post-moult, the total titratable base and the total apparent  $\text{H}^+$  flux were in quite close agreement, as shown in Table 2.

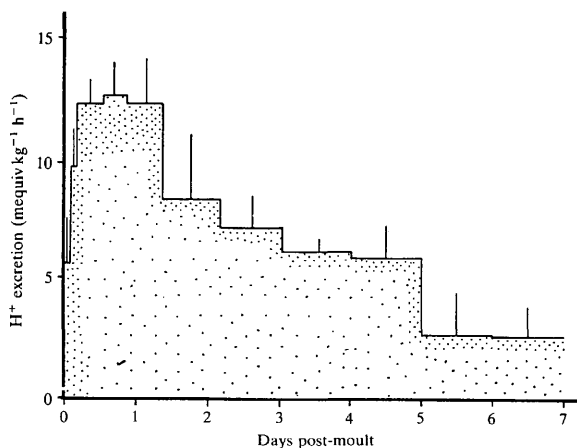


Fig. 4. The apparent  $H^+$  excretion rates for nine crabs as a function of time after the moult. The vertical bars show  $\pm 1$  s.e. for each time interval.

The total  $CO_2$  pools in the intermoult crab were estimated from the carapace and whole carcass titrations as outlined in Methods, and for the blood and intracellular fluid from data collected in acid-base studies (Wood & Cameron, 1985). For the blood and intracellular fluids, the total  $CO_2$  pool sizes were calculated as the concentration times pool size, and for the carapace, as the total titratable base content of the acid-extracted material minus the phosphate contribution. The results of these pool size calculations are given in Table 3, showing that the  $CO_2$  pool of the carapace dwarfs the other body pools. A reasonable estimate of the  $CO_2$  excretion rate of intermoult animals at  $25^\circ C$  is about  $1.5 \text{ mmol kg}^{-1} \text{ h}^{-1}$  (Fig. 5 and unpublished data), which means that the carapace represents roughly 29 days'  $CO_2$  excretion at normal metabolic rates. Since the carapace appears to be mineralized at a rate greater than that, we made measurements to determine whether an elevated metabolic rate or  $CO_2$  uptake from the environment served as the source of  $CO_2$  in the carbonates.

Table 2. The total titratable base in six post-moult crabs compared with the total apparent  $H^+$  flux observed from just after moult until the crab was killed

Crab	Total base	Total $H^+$ flux	Ratio
A	0.525	0.457	1.15
B	1.316	1.059	1.24
C	0.995	0.914	1.09
D	0.678	0.607	1.11
E	0.880	1.062	0.83
F	1.179	1.162	1.01
Mean $\pm$ s.e.			$1.07 \pm 0.14$

The crabs range from 3 to 7 days post-moult.



The data for  $O_2$  consumption and  $CO_2$  excretion following moult for five crabs are shown in Fig. 5. During the first 5 days following moult, the crabs had an elevated rate of  $O_2$  consumption, about twice the intermoult value at first and declining gradually. The crabs consistently had a net negative  $CO_2$  excretion rate, i.e. a net  $CO_2$  uptake from the sea water. Based on the assumption that the metabolic RQ during the post-moult period would have a normal value of 0.9, a 'net  $CO_2$  deficit' was calculated as follows: the  $O_2$  consumption was multiplied by 0.9, the observed  $CO_2$  excretion subtracted from that (sign observed), and the result multiplied by 2 (to convert from moles to equivalents). As shown in Table 4, the equivalent net  $CO_2$  deficit matches quite satisfactorily with the observed apparent  $H^+$  excretion, showing that the source for the  $CO_2$  in the carapace must be a combination of metabolic  $CO_2$  and  $CO_2$  from the environmental sea water.

A preliminary experiment was performed to try to determine whether pH or  $HCO_3^-$  concentration of the sea water most influenced the observed rate of mineralization. Depressing the sea water pH from 8.15 to 6.4 by titration with HCl reduced the  $HCO_3^-$  concentration from 1.85 to 0.07 mequiv  $l^{-1}$ , and reduced the apparent  $H^+$  flux from +7.1 to -2.4 mequiv  $kg^{-1} h^{-1}$ . Subsequent increase of the  $P_{CO_2}$  from < 0.3 to 15 Torr decreased the pH slightly more, to 6.35, but restored the sea water  $HCO_3^-$  concentration to 1.79 mequiv  $l^{-1}$ , and the apparent  $H^+$  flux to +12.6 mequiv  $kg^{-1} h^{-1}$ . This would seem to indicate a greater dependence upon  $HCO_3^-$  gradients than upon  $H^+$  gradients, although the interpretation may be complicated by the internal hypercapnic acidosis which would also result.

#### *The effects of acetazolamide*

The apparent  $H^+$  excretion rates for six post-moult crabs treated with acetazolamide are given in Fig. 6, along with the data from Fig. 4 as controls. There was a highly significant reduction of the apparent  $H^+$  rate, especially during the first 36 h after moulting, when the treated values reached only 30–40% of the control value. In individual crabs, the effect appeared to wear off after about 24 h, when rates began to return toward the control values.

## DISCUSSION

### *The intermoult carapace*

The composition of the intermoult carapace reported here largely confirms similar data for the blue crab (Vigh & Dendinger, 1982) and for other crustaceans (Kleinholz, 1941; Lafon, 1943). That is, the predominant mineral component is  $CaCO_3$ , with lesser contributions of  $Mg^{2+}$  and  $PO_4^{-3}$  and traces of  $Si^{2+}$ . In our analyses, there was a small but significant weight contribution of  $Na^+$  and  $K^+$ , which were probably trapped in the mineral matrix, along with  $Cl^-$ . As with other skeletal mineral deposits, there is probably some water of hydration associated with the crystal structure, which would account for part of the weight loss upon acid extraction. Others have also studied the various proportions of water- and acid-soluble protein bound in the mineral matrix (Welinder, 1975).

We are not aware of any previous report of the substantial water compartment of the carapace, however, nor have any previous studies focused on the acid-base status

Table 3. Comparison of the total CO<sub>2</sub> pools in intermoult blue crabs per kg body weight

Total intracellular CO <sub>2</sub>	0.73 mmol
Total extracellular CO <sub>2</sub>	1.07 mmol
Sum	1.80 mmol
Total carapace CO <sub>2</sub>	1043 mmol
Ratio 1043/1.80 =	579

The values for the intracellular and extracellular pools are derived from measurements of the compartment volumes and concentrations in Wood & Cameron (1985).

The values for the shell compartment are from Table 1 and Fig. 1.

of this compartment. In reviews of the chemistry of calcium in biological fluids, it has been pointed out that calcium is normally at supersaturating levels in physiological fluids, and that maintenance of a solid CaCO<sub>3</sub> phase would require a more alkaline pH than that of the blood (Burton, 1976; Campbell & Boyan, 1974). Our studies confirm that the carapace fluid compartment is maintained at a pH 0.3 to 0.4 units more alkaline than blood. (For further details on the intracellular pH measurements see Wood & Cameron, 1985.) Some additional confirmation of this may be found in a study of two other crabs, albeit inadvertently. Cameron (1981) used the DMO technique to study the 'mean whole body' intracellular pH in two species of land crabs (*Birgus latro* and *Cardisoma carnifex*). This method entails an assumption of uniform intracellular pH. The values he obtained were only about 0.15 units below the blood pH, rather than the 0.5 units that were expected on the basis of studies on single muscle cells (Hinke & Menard, 1976). The upward bias in

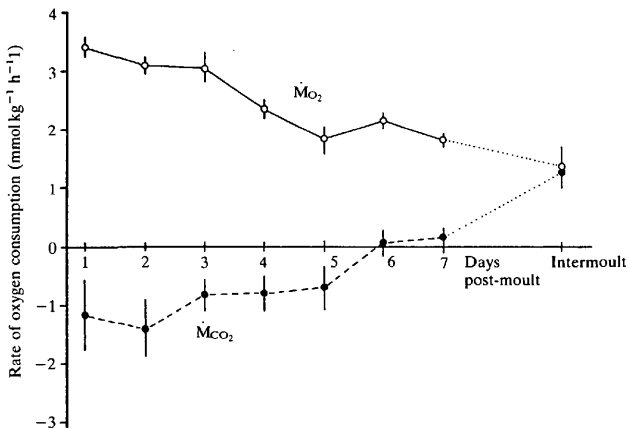


Fig. 5. Mean rates of oxygen consumption ( $\dot{M}_{O_2}$ ) and CO<sub>2</sub> excretion ( $\dot{M}_{CO_2}$ ) for six crabs as a function of time following completion of the moult, and for three intermoult crabs. The vertical bars represent  $\pm 1$  s.e.

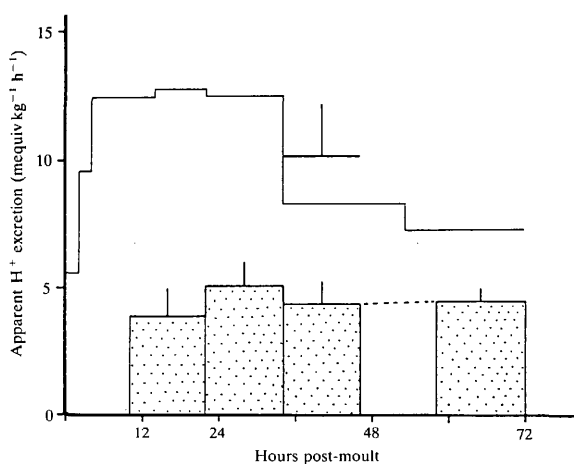


Fig. 6. The effects of  $10^{-4}$  M-acetazolamide injection on apparent  $H^+$  excretion for six crabs. Three crabs were injected during the first 12 h after moult; three others were used as additional controls (dark horizontal bar) then treated and studied through day 3 post-moult. The solid line across the top is a repeat of control data from Fig. 4.

the mean whole body estimate is quite satisfactorily accounted for by a relatively alkaline shell compartment comprising 14% of the total body water. This alkaline compartment will no doubt prove of further interest in investigations of the role of the carapace in chronic and acute acid-base buffering, since the carapace has already been implicated in hypercapnia (Henry, Kormanik, Smatresk & Cameron, 1981), emersion (DeFur, Wilkes & McMahon, 1980) and exercise (Wood & Randall, 1981).

#### *The shed carapace*

Various statements in the literature imply that there is considerable resorption of material from the carapace prior to moulting (e.g. Roer, 1980; Passano, 1960). There were no apparent differences in the inorganic constituents of the intermoult and shed carapaces, but our data do indicate a resorption of perhaps half of the organic materials. A rise in the blood calcium concentration is sometimes cited as evidence for mineral resorption prior to the moult (Robertson, 1937; Roer, 1980), but some simple calculations show that this aspect must be trivial. The total calcium pool of the intermoult crab is 37.3 g, or  $0.93 \text{ mol kg}^{-1}$  (Table 1; Fig. 1), and the blood calcium pool only  $0.002 \text{ mol kg}^{-1}$  (using a blood volume of  $245 \text{ ml kg}^{-1}$  and calcium concentration of  $8.6 \text{ mmol l}^{-1}$ ; Wood & Cameron, 1985). Dissolution of only 1% of the carapace pool in the blood would increase the blood concentration 5.5-fold, far more than has been observed pre-moult (cf. Robertson, 1960; D. W. Towle & C. P. Mangum, personal communication). Any significant dissolution of the carapace  $\text{CaCO}_3$  pool must be accompanied by a rapid shuttle of the  $\text{Ca}^{2+}$  to the external sea water, since the blood is simply an inadequate reservoir for  $\text{Ca}^{2+}$ .

Table 4. Mean values for the calculated 'net CO<sub>2</sub> deficit' and apparent H<sup>+</sup> flux for five post-moult and three intermoult (IM) crabs

Day	CO <sub>2</sub> deficit (mequiv kg <sup>-1</sup> h <sup>-1</sup> )	Apparent H <sup>+</sup> flux (mequiv kg <sup>-1</sup> h <sup>-1</sup> )	Ratio
1	8.49	10.07	0.84
2	8.37	9.16	0.91
3	7.08	10.14	0.70
4	5.80	6.46	0.90
5	4.62	5.50	0.84
6	3.75	4.64	0.81
7	2.98	2.81	1.06
Intermoult	-0.05	0.06	-

The CO<sub>2</sub> deficit is calculated on the assumption that the metabolic RQ is 0.9; see text for details.  
The ratio is the CO<sub>2</sub> deficit divided by the apparent H<sup>+</sup> flux.

storage. The tissues are no more likely to serve this storage function, since intracellular free Ca<sup>2+</sup> levels are generally very low. Some dissolution of the carapace along suture lines is probably important in enabling the moult to occur, but the resulting Ca<sup>2+</sup> ions are probably eliminated *via* the gills. Additional support is provided by the almost negligible Ca<sup>2+</sup> content of the immediately post-moult crabs (Fig. 3).

#### Calcification following the moult

For most crustaceans the pattern of calcification is similar, with a rapid uptake and deposition in the first few days, later slowing and continuing for some further period. The lag period immediately after ecdysis may be as long as 2 days (Welinder, 1975), but it was only a few hours in our study, which matches the patterns previously reported for *Callinectes sapidus* by Vigh & Dendinger (1982) and Greenaway (1983). The maximum rate of uptake reported by Greenaway was only 1.3 mmol kg<sup>-1</sup> h<sup>-1</sup>, however, whereas the rate implied by our Figs 3 and 4 is almost five times as high.

During this period of maximal uptake, the blood Ca<sup>2+</sup> concentration reportedly drops (Robertson, 1960; D. W. Towle & C. P. Mangum, personal communication), but these reports should perhaps be re-examined. Based upon estimates of cardiac output for the blue crab at 25 °C (Mangum & Weiland, 1975) and the maximal Ca<sup>2+</sup> rates above, the arterio-venous difference might be 1 mmol l<sup>-1</sup> or more, so if venous sampling sites were used (as is common), the arterial (or post-gill) blood may be closer to the intermoult values.

#### Apparent H<sup>+</sup> excretion and CO<sub>2</sub> dynamics

A number of terms have been employed in the literature to denote apparent acid or base excretion across the gills of aquatic animals. In the usual case, what is actually measured is a change in the acid-base status of the external milieu, which may appear as acidification or alkalization. A decrease in the external titratable alkalinity may occur by excretion of a proton, by uptake of OH<sup>-</sup> ion, by uptake of HCO<sub>3</sub><sup>-</sup>, etc. To use the term 'acid excretion' in this context is both incorrect and

misleading. Incorrect because 'acid' implies an identified anion or conjugate base, and misleading because no excretion need occur. We have therefore employed the term 'apparent  $H^+$  excretion' to acknowledge these difficulties.

It is true that the formation of  $CaCO_3$  entails the disposal of acidic equivalents, if not actually protons, according to equation 1, and the data show a satisfactory correspondence between the apparent  $H^+$  excretion and the rate of calcification (Figs 3, 4). The average maximum of  $12.5 \text{ mequiv kg}^{-1} \text{ h}^{-1}$  may not be exceptional for post-moult crabs, since we also measured maximum rates of  $11\text{--}12.4 \text{ mequiv kg}^{-1} \text{ h}^{-1}$  in a stone crab (*Menippe mercenaria* Say) that moulted in the laboratory. These rates are extremely high compared to what has been observed in other acid-base studies of aquatic animals. The highest previously recorded rate for a crab is about  $2 \text{ mequiv kg}^{-1} \text{ h}^{-1}$  following an emersion hypercapnia in *Carcinus maenas* (Truchot, 1979) and in *Callinectes sapidus* following aquatic hypercapnia (J. N. Cameron, unpublished data). For fish the maximum rates are even lower, up to  $1.1 \text{ mequiv kg}^{-1} \text{ h}^{-1}$  during hypercapnia (Heisler, 1982). Compared on an area basis, using published data for gill area of the blue crab (Aldridge & Cameron, 1982), the apparent  $H^+$  excretion rate is  $1.7 \mu\text{equiv cm}^{-2} \text{ h}^{-1}$ , several times that of frog gastric mucosa (Obrink, Waller & Berglinde, 1978), an order of magnitude greater than that of turtle bladder (Steinmetz, 1967) and about half the rate reported for histamine-stimulated piglet gastric mucosa (Ekblad, Machen, Licko & Rutten, 1978). Some flux may occur across the general body surface, but since the gills constitute the bulk of the external surface, there would be little difference if the calculations included all surfaces.

These high rates of  $Ca^{2+}$  uptake and apparent  $H^+$  excretion must be accompanied by an equivalent transport of  $CO_2$  to the deposition sites, as pointed out earlier. The carbonates of the carapace represent such a large reservoir of  $CO_2$  that it was difficult *a priori* to say where it came from. One alternative would be a greatly elevated rate of metabolism, which the data of Fig. 5 show did not occur. The only alternate source of  $CO_2$  is the small amount of physically dissolved  $CO_2$  in the sea water, and the much greater reservoir of  $HCO_3^-$ . The data of Fig. 5 show clearly that the net  $CO_2$  excretion was negative for some days following the moult, which confirms the external source. There was also good agreement between the calculated  $CO_2$  deficit and the observed apparent  $H^+$  excretion; the expected ratio, based on the proportion of carbonate and phosphate (Table 1) was 0.94, whereas the mean ratio from Table 4 was 0.87.

There appear, however, to be two different mechanisms by which  $CO_2$  could be entering from the sea water (Fig. 7). One alternative, shown on the right, involves an outward transport of  $H^+$  ions. The acidification of the sea water in the gills would lead to a redistribution of  $CO_2$  and  $HCO_3^-$ , such that the dissolved  $CO_2$  would rise. This could presumably provide a diffusive gradient leading to net  $CO_2$  movement into the blood through the gills. The flaw in this scheme is that the residence time for water in the blue crab's gills is only about 6 s (Cameron, 1979), and the uncatyalsed rate of dehydration of  $HCO_3^-$  is too slow to allow any significant rise in the  $P_{CO_2}$  before the water leaves the contact region (Edsall, 1969). The other alternative, which seems to be favoured by the evidence at present, is drawn on the left and involves a direct  $HCO_3^-$  uptake mechanism. Our preliminary work has

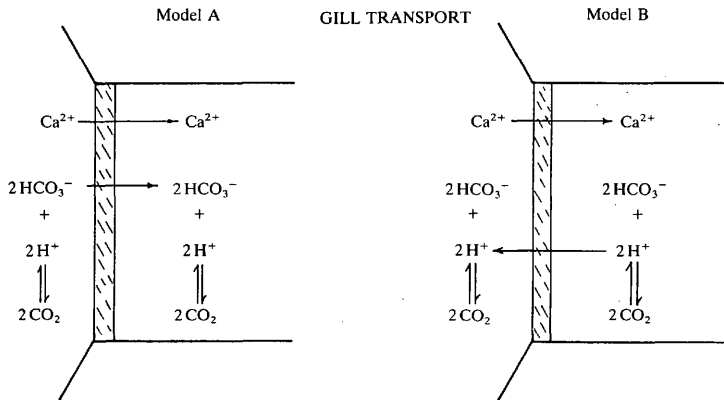


Fig. 7. Theoretical model of possible  $\text{Ca}^{2+}$  and  $\text{HCO}_3^-$  uptake schemes. See text for discussion.

provided tentative support for this mechanism, since the apparent  $\text{H}^+$  excretion was reversed by a reduction of sea water pH and  $\text{HCO}_3^-$  concentration, then restored by increasing bicarbonate without increasing pH. The significant reduction in apparent  $\text{H}^+$  excretion caused by acetazolamide treatment suggests that carbonic anhydrase is important in this overall process (cf. Giraud, 1981), but does not give any clues as to whether the effect is exerted directly upon transport across the gills or epithelium, or indirectly *via* acid-base disturbances in blood and/or tissue.

The only other report of which we are aware that shows negative  $\text{CO}_2$  excretion in an aquatic animal is a brief report by Dejours (1969) claiming reversal of  $\text{CO}_2$  excretion after transfer of a fish to  $\text{Cl}^-$ -free water. Dejours & Beekenkamp (1978) also reports acid-base disturbances in crayfish during the moult cycle.

The transport of  $\text{Ca}^{2+}$ ,  $\text{CO}_2$  and other ions across the carapace epithelium is a matter of equal interest, but little information is currently available. It seems clear from the blood and carapace pH data that there must be an active  $\text{H}^+$  ion pump, possibly linked with some other ion ( $\text{Na}^+$ ?), as well as  $\text{Ca}^{2+}$  and possibly  $\text{HCO}_3^-$  transport mechanisms. The diffusive gradients for dissolved  $\text{CO}_2$  across both the gills and the other surfaces will be interesting subjects of further study.

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