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THE ROLE OF $CaCO_3$ DISSOLUTION AS A SOURCE OF HCO_3^- FOR THE BUFFERING OF HYPERCAPNIC ACIDOSIS IN AQUATIC AND TERRESTRIAL DECAPOD CRUSTACEANS

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SUMMARY

Blood calcium concentrations are elevated during a hypercapnic acidosis in the terrestrial crab *Gecarcinus lateralis*, but not in the aquatic *Callincetes sapidus*. The increase occurs concomitantly with a rise in blood HCO_3^- and partial restoration of resting blood pH values. It is believed that in *G. lateralis* that a source of $CaCO_3$, possibly the shell, is being dissolved for buffering purposes.

INTRODUCTION

Both aquatic and terrestrial animals respond to a respiratory acidosis by increasing the levels of blood HCO_3^- to bring blood pH back toward the normal resting value (Cameron & Randall, 1972; Reeves, 1977). In water-breathing animals there is evidence that the mechanisms responsible for controlling blood HCO_3^- are branchial ion exchanges (Cl^- for HCO_3^- and/or Na^+ for H^+). This work has been done on teleost fish (Maetz & Garcia-Romeu, 1964; Randall & Cameron, 1973; Cameron, 1976) and decapod crustaceans (Krogh, 1938; Truchot, 1975, 1979; Cameron, 1978) as well as other invertebrates. Air-breathing vertebrates are known to employ ventilatory control of P_{CO_2}, HCO_3^- reabsorption via the kidney and mobilization of $CaCO_3$ in bone (Davenport, 1974), for pH compensation.

The terrestrial decapod crustaceans are interesting because although they show a ventilatory response to increased CO_2 (Cameron & Mecklenburg, 1973; Cameron, 1975) their mechanism for long-term acid–base regulation is unknown. They lack the opportunity for branchial ion exchange available to aquatic crabs, and in aquatic crabs the antennal gland apparently does not play a role in acid–base balance (Cameron & Batterton, 1978; Truchot, 1975, 1979). Fully terrestrial crabs do attempt to compensate a hypercapnic respiratory acidosis by raising blood HCO_3^- (Cameron, 1981) but where the HCO_3^- originates is not known.

Since it has been shown that some other organisms, bivalve molluscs in particular, utilize $CaCO_3$ as a source of HCO_3^- to buffer pH disturbances in the blood (Dugal,

1939; Mangum, Henry & Simpson, 1979) it is reasonable to suggest that terrestrial crabs may do the same. We have investigated that possibility by following the concentrations of blood Ca in a terrestrial and an aquatic crab during hypercapnic acidosis.

MATERIALS AND METHODS

Adult intermoult individuals of *C. sapidus* and *G. lateralis* were collected respectively by trawl from Lydia Ann Channel, Port Aransas, Texas, and by hand from the back dunes of Boca Chica, Texas. *C. sapidus* were held in 500 l aerated, running seawater tanks at 900 mOsm/kg and 26 °C. *G. lateralis* were maintained in sand-filled tubs at 25 °C provided with dechlorinated tap water and fed on dry dog food, lettuce, and melon. Animals were kept for from 2 to 30 days before use.

Blue crabs weighing between 200 and 300 g were put in a flow-through chamber, the water either being normocapnic or being made hypercapnic (P_{CO_2} = 15 torr) by equilibration in a stripping column with a mixture of CO_2 in air provided by a Wösthoff gas-mixing pump. *G. lateralis* (80–100 g) were placed in individual plexiglas chambers into which either humidified air or 3% CO_2 in humidified air was pumped. About 5 ml of 80% seawater were placed in each chamber to prevent desiccation.

Blood was withdrawn from the infrabranchial sinus using a 22 gauge needle and syringe. P_{CO_2} and pH were measured immediately on a Radiometer-Copenhagen PHM 71 acid-base analyser and water-jacketed (T = 23 °C for *G. lateralis* and 26 °C for *C. sapidus*) pH and P_{CO_2} electrodes (Radiometer G297 and E503610). The total CO_2 was measured using a conductometric method, which depends upon conversion of all combined forms (bicarbonates, etc.) to dissolved CO_2 gas by acidification, removal in a carrier gas stream, followed by absorption in alkali and detection by differential conductivity (Maffly, 1968; Cameron, unpublished). For the smaller *G. lateralis* the P_{CO_2} was calculated from the Henderson–Hasselbalch equation:

$$\mathrm{pH} = \mathrm{pK} + \log\left(\frac{C_T}{\alpha . P_{CO_2}} - 1\right)$$

using a pK value of 6·073 and α of 0·0409 from Smatresk, Preslar & Cameron (1979). The amount of HCO_3^- which arose passively from the elevation of P_{CO_2} was calculated from an HCO_3^-/pH (Davenport, 1974) diagram using the non-bicarbonate *in vitro* blood buffer value (β) of 16 slykes from Smatresk *et al.* (1979). Base excess (BE) for each serially sampled animal, then, was calculated as the difference between the actual measured [HCO_3^-] and the [HCO_3^-] predicted from the buffer line.

The remaining blood was allowed to clot in the syringe; the clot was then disrupted and the blood centrifuged. Calcium concentrations were determined on an atomic absorption spectrophotometer (Perkin Elmer 303). The blood osmolality was measured using a vapour-pressure osmometer (Wescor Model 5130B).

RESULTS

When exposed to hypercapnia both species exhibited a typical respiratory acidosis and compensation. In *Callinectes* the blood pH dropped 0·3 unit over the first ho

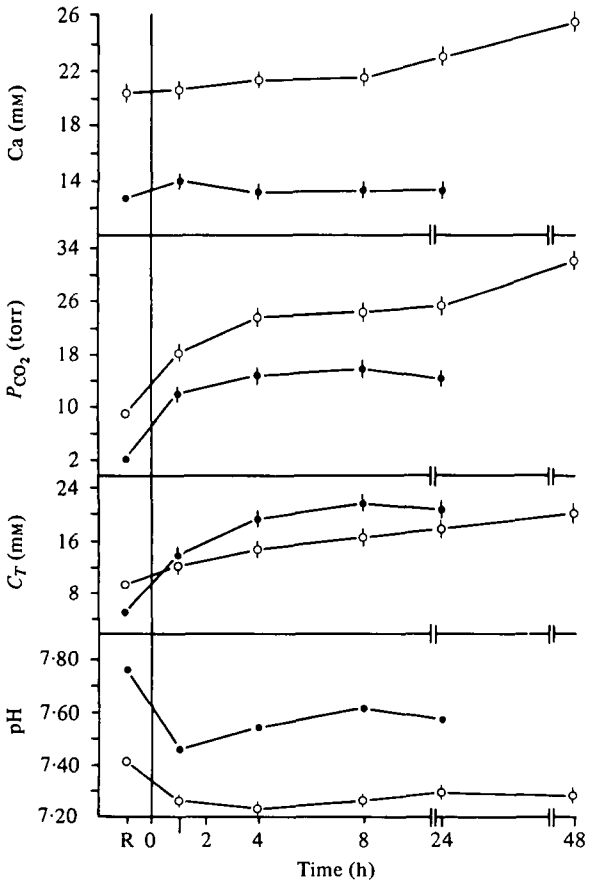

Fig. 1. Time course of blood Ca, P_{CO_2}, C_T and pH in *Callinectes sapidus* (closed circles) and *Gegarcinus lateralis* (open circles) at rest (*R*) and during hypercapnia. Where standard error bars are not shown, the S.E., was smaller than the size of the circle used to represent the data point. T = 23–26 °C. For *Callinectes* N = 21–33 (resting) and N = 10 (hypercapnia). For *Gecarcinus* N = 24 (resting) and N = 12 (hypercapnia).

and then rose approximately 0·15 unit to a partially compensated value of 7·61 by 8 h (Fig. 1). No further compensation occurred beyond 8 h. The partial restoration of normal (resting) blood pH was accomplished by a significant ($P < 0{\cdot}05$, F test) elevation of blood HCO_3^- between 1 and 8 h of hypercapnia (Fig. 1). During both the uncompensated and the compensated phases of the acidosis, the blood Ca concentration did not change significantly from the resting value (Fig. 1; $P > 0{\cdot}05$, F test).

G. lateralis underwent a less severe acidosis, with the pH dropping about 0·18 unit during the initial 4 h of hypercapnia (Fig. 1). However, compensation was slower and less complete than in *C. sapidus*. By 24 h the pH had risen only about 0·07 unit to a value of 7.30, and there was no further increase in HCO_3^- or pH between 24 and 48 h. The major point of interest is that the mean blood Ca concentration increased significantly during the period of compensation, and the Ca concentration increased for every individual crab (Fig. 1; $P < 0{\cdot}01$, paired t test). From a resting value of 10·45 mM, the mean Ca concentration increased by about 3 mM at 24 h, and by

Table 1. *Base excess and the rise in blood calcium levels for each of* 12 Gecarcinus lateralis *exposed to* 3% CO_2 *for* 24 *h at* 25 °C

Crab	Base excess (mM HCO_3^-)	Δ Ca^{2+} (mM)
1	7·28	1·01
2	5·70	0·91
3	5·59	1·15
4	3·66	1·87
5	5·16	2·01
6	3·28	2·56
7	6·60	6·51
8	4·51	3·23
9	5·47	7·10
10	4·25	3·96
11	9·21	2·66
12	8·09	2·18
Mean	5·82	2·93
S.E.	±0·52	±0·58

almost 6 mM at 48 h. The relationship between base excess (BE) and the increase in Ca for individual crabs was, unfortunately, variable, so no good estimate of the stoichiometry could be made. Both BE and Ca increased in every individual, however, and the ratio of means was roughly 2:1 (BE to ΔCa; Table 1).

The blood osmolality did not vary more than 2% from the resting mean of 976 ± 11 mOsm/kg in *G. lateralis* during the experiments, and the mean values at 24 h and 48 h were not significantly different from those at rest.

DISCUSSION

The responses of the blood acid–base parameters to hypercapnic acidosis in both *C. sapidus* and *G. Lateralis* were typical of crabs, and of water-breathers in general (see review by Reeves, 1977). The rapid and relatively large compensation seen in *C. sapidus* agrees well with published data on freshwater-adapted individuals of this species (Cameron, 1978); and the relatively slow and small compensation observed in *G. lateralis* agrees fairly well with data from *Carcinus maenas* in air (Truchot, 1975) and the terrestrial coconut crab *Birgus latro* (Cameron, 1981). The two species' responses were quite different in one significant aspect, however; there was no change at all in the Ca concentration in *C. sapidus*, and a significant rise in every individual of *G. lateralis*.

The data for the aquatic *C. sapidus* are consistent with the current theory, which holds that ionic exchanges between blood and water, usually via the gills, are responsible for the maintenance of pH in aquatic animals. For example, Cameron (1976, 1978) found changes in the relative rates of Na and Cl fluxes in response to hypercapnia that were consistent with (hypothesized) changes in Na^+/H^+ and Cl^-/HCO^-_3 exchanges that would regulate pH in response to hypercapnic acidosis. De Renzis & Maetz (1973) and others have reported a link between ionic exchange and blood pH in fish. Truchot (1975, 1979) has reported large branchial acid/base fluxes in response to various disturbances.

Ionic exchange in the gills is clearly not an option for *G. lateralis*, nor for any fully terrestrial crab. Neither is the antennal gland likely to provide the needed route for acid excretion, since studies done on antennal glands have shown either no significant acidification capability (Cameron & Batteron, 1978) or very low flow (Harris, 1977), or both. Since compensation does occur, as shown in Fig. 1 and Table 1, The most likely source appears to be the large $CaCO_3$ reservoir of the shell, which will yield Ca^{2+} and HCO_3^- upon acidification. The observed increases in Ca and HCO_3^- concentrations were independent of any change in blood osmolality, so the results were not a spurious consequence of dehydration.

The shell has been implicated in the buffering of pH disturbances in other organisms: Dugal (1939) reported a visible erosion of the shell, along with elevated mantle fluid Ca^{2+} and HCO_3^- concentrations during several days of anaerobiosis in the bivalve *Venus mercenaria*; and a similar rise in Ca^{2+} and HCO_3^- was seen during valve closure in the bivalve *Rangia cuneata* (Magnum *et al.* 1979). These water-breathing molluscs appear to utilize their shells only during periods of valve closure, when they are cut off from contact with the large ion pool in the ambient sea water.

Not surprisingly, terrestrial gastropod molluscs exhibit the same pattern of elevation of blood Ca_2^+ and HCO_3^- during hypercapnia. Burton (1976), in discussing the appearance of Ca^{2+} and HCO_3^- in *Helix pomatia* during hypercapnia, proposed that the ions could originate either from acidification of $CaCO_3$ in the shell, or from bound intracellular Ca^{2+}. If the origin of the Ca^{2+} is the shell, then a 1:1 correspondence between BE and ΔCa^{2+} would be expected. If the mechanism is replacement of bound intracellular Ca^{2+} by H^+ ions, the same relationship would be expected, so the present study does not allow a choice. Intracellular Ca^{2+} in crustaceans is low, however, and it would have to be virtually exhausted to account for the 3–6 mM rise seen in the blood of *G. lateralis*.

Regardless of the variability of the data for *G. lateralis* (Table 1), which is distressing, the argument for $CaCO_3$ dissolution for buffering purposes remains strong. The increase in blood Ca occurs only in the terrestrial species in which acid–base regulation via branchial ion exchange is not an option. The increase begins during the period of compensatory pH adjustment, 8–24 h (Fig. 1), and continues through 48 h, during which time blood pH remains stable despite an increase in the P_{CO_2}. The relative times of compensation in *G. lateralis* and *C. sapidus* indicate that a much slower process is occurring in the former: this is compatible to the idea of the slow dissolution of $CaCO_3$ by a weak acid (H_2CO_3) as compared to the more rapid ion exchange mechanisms of *C. sapidus*. We believe the most obvious source of $CaCo_3$ is the shell, although other sources such as $CaCO_3$ granules in digestive gland (Becker *et al.* 1974) cannot be ruled out.

How important the source of $CaCO_3$ is in nature has yet to be determined. In most cases an increase in ventilation is probably adequate to relieve internal acidosis brought about by exercise, for example (Smatresk *et al.* 1979). Only when the ventilatory control mechanism is insufficient to restore pH, such as in prolonged hypercapnia or exhaustion, or during temperature variation, might $CaCO_3$ become important in acid–base regulation.

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REFERENCES

BECKER, G. L., CHEN, C., GREENAWALT, J. W. & LEHNINGER, A. L. (1974). Calcium phosphate granules in the hepatopancreas of the blue crab *Callinectes sapidus*. *J. Cell. Biol.* **61**, 316–326.

BURTON, R. F. (1976). Calcium metabolism and acid–base balance in *Helix pomatia*. In *Perspectives in Experimental Biology* (ed. P. Spencer Davies), pp. 7–16. New York: Academic Press.

CAMERON, J. N. (1975). Aerial gas exchange in the terrestrial Brachyura *Gecarcinus lateralis* and *Cardisoma guanhumi*. *Comp. Biochem. Physiol.* **31** (4), 632–634.

CAMERON, J. N. (1976). Branchial ion uptake in arctic grayling: resting values and effects of acid–base disturbance. *J. exp. Biol.* **64**, 711–725.

CAMERON, J. N. (1978). Effects of hypercapnia on blood acid–base status, NaCl fluxes, and trans-gill potential in freshwater blue crabs, *Callinectes sapidus*. *J. comp. Physiol.* **123**, 137–141.

CAMERON, J. N. (1981). Acid–base responses to changes in CO_2 in two Pacific crabs: the coconut crab, *Birgus latro*, and a mangrove crab, *Cardisoma carnifex*. *J. exp. Zool.* (submitted).

CAMERON, J. N. & BATTERTON, C. V. (1978). Antennal gland function in the freshwater blue crab, *Callinectes sapidus*: water, electrolyte, acid–base and ammonia excretion. *J. comp. Physiol.* B, **123**, 143–148.

CAMERON, J. N. & MECKLENBURG, T. A. (1973). Aerial gas exchange in the coconut crab, *Birgus latro*, with some notes on *Gecarcoidea lalandi*. *Resp. Physiol.* **19**, 245–261.

CAMERON, J. N. & RANDALL, D. J. (1972). The effect of increased ambient CO_2 on arterial CO_2 tension, CO_2 content and pH in rainbow trout. *J. exp. Biol.* **57**, 673–680.

DAVENPORT, H. W. (1974). *The ABC of Acid–Base Chemistry*, 6th edition, 125 pp. Chicago: University of Chicago Press.

DE FUR, P. L., WILKES, P. R. H. & MCMAHON, B. R. (1980). Non-equilibrium acid–base status in *C. productus*: role of exoskeletal carbonate buffers. *Resp. Physiol.* **42**, 247–261.

DERENZIS, G. & MAETZ, J. (1973). Studies on the mechanism of chloride absorption by the goldfish gill: relation with acid–base regulation. *J. exp. Biol.* **59**, 339–358.

DUGAL, C. P. (1939). The use of calcareous shell to buffer the product of anaerobic glycolysis in *Venus mercenaria*. *J. Cell. comp. Physiol.* **13**, 235–251.

HARRIS, R. R. (1977). Urine production rate and water balance in the terrestrial crabs *Gecarcinus lateralis* and *Cardisoma guanhumi*. *J. exp. Biol.* **68**, 57–67.

KROGH, A. (1938). The active absorption of ions in some freshwater animals. *Z. Vergl. Physiol.* **25**, 335–350.

MAETZ, J. & GARCIA-ROMEU, F. (1964). The mechanism of sodium and chloride uptake by the gills of freshwater fish *Carassius auratus*. II. Evidence for NH_4^+/Na^+ and HCO_3^-/Cl^- exchange. *J. gen. Physiol.* **47**, 1209–1226.

MAFFLY, R. H. (1968). A conductometric method for measuring micro-molar quantities of carbon dioxide. *Anal Biochem. exp. Med.* **23**, 252–262.

MANGUM, C. P., HENRY, R. P. & SIMPSON, D. M. (1979). The effect of ouabain on blood NaCl in the osmoregulating clam *Rangia cuneata*. *J. exp. Zool.* **207**, 329–335.

RANDALL, D. J. & CAMERON, J. N. (1973). Respiratory control of arterial pH as temperature changes in the rainbow trout *Salmo gairdneri*. *Am. J. Physiol.* **225**, 997–1002.

REEVES, R. B. (1977). The interaction of body temperature and acid–base balance in ectothermic vertebrates. *A. Rev. Physiol.* **39**, 559–586.

SMATRESK, N. J., PRESLAR, A. J. & CAMERON, J. N. (1979). Post-exercise acid–base disturbance in *Gecarcinus lateralis*, a terrestrial crab. *J. exp. Zool.* **210**, 205–210.

TRUCHOT, J. P. (1975). Blood acid–base changes during experimental emersion and reimmersion of the intertidal crab, *Carcinus maenas*. *Resp. Physiol.* **23**, 351–360.

TRUCHOT, J. P. (1979). Mechanisms of the compensation of blood respiratory acid–base disturbances in the shore crab, *Carcinus maenas*. *J. exp. Zool.* **210**, 407–416.