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Physiological Responses to Air Exposure: Acid-Base Balance and the Role of Branchial Water Stores[1]

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SYNOPSIS. Intertidal organisms usually exhibit one of two behaviors when they are air exposed. They either isolate themselves from the aerial environment or they interact with the aerial environment. Among the animals using the first behavior, body fluid acid-base balance is partially maintained by dissolution of the calcium carbonate shell in the bivalve molluscs to buffer the metabolic acids produced anaerobically. Calcium ions compensate for the acidosis by increasing the strong ion difference. The release of carbonate from the shell causes an increase in molecular CO_2 which offsets the compensation somewhat, but this effect is minimized by distributing the CO_2 among the other fluids within the shell and/or by venting the shell to the air. In animals which have a fairly high concentration of a respiratory pigment, such as a lugworm, an anaerobically induced acidosis is minimized by a large Haldane effect. Among the animals which interact with the aerial environment, the decapod crustaceans by and large maintain their metabolism aerobically, although it may be greatly reduced. A respiratory acidosis due to elevated hemolymph P_{CO_2} may be either fully compensated or not at all. Compensation involves an increase in hemolymph calcium, probably from the calcium carbonate exoskeleton. Compensatory mechanisms may also include branchial water stores which accumulate a titratable base. It is suggested that the alkalinization of the branchial water maintains a steeper P_{CO_2} gradient across the gill and reduces the magnitude of the acidosis for a short period of time. The ability to use branchial water stores in this way may be tied to the ability of the animal to osmoregulate.

INTRODUCTION

Intertidal organisms experience alternating periods of exposure to air and water, the duration of which depends on the organism's mobility, its position in the intertidal zone, and the amplitude of the tide. When confronted with exposure to air (emersion), an organism will exhibit one of two overall behaviors: it may (1) isolate itself from the aerial environment by burrowing into the substratum or by closing its shell completely as in the case of a clam, or it may (2) continue to interact with its aerial surroundings to some degree. The two behaviors are interesting because the organisms which isolate themselves from the air also isolate themselves from a source of oxygen and, therefore, they invariably switch from a predominantly aerobic metabolism to a predominantly anaerobic metabolism. Organisms which continue to use air as a respiratory medium must solve problems associated with the important differences between air and water as a medium for exchanging both oxygen (deFur, 1988) and carbon dioxide. Both strategies are interesting from the perspective of acid-base balance. On the one hand, anaerobic metabolism results in the production of various types of metabolic acids in different organisms (de Zwaan, 1983). Alternatively, air breathers hypoventilate their respiratory surfaces relative to water breathers because of the much larger O_2 capacitances of air (Dejours, 1975). This relative hypoventilation results in the retention of CO_2, causing a respiratory acidosis when the medium switches from water to air.

The purpose of this article is to review the changes in acid-base status which are known to occur in intertidal animals during air exposure and to discuss the mechanisms of compensation relating specifically to changes in the blood acid-base balance. In the sections below, the overall mechanisms of adaptation to an intertidal environment are addressed using representative groups of organisms where something is known of the acid-base response of the body fluids.

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ISOLATION FROM THE AERIAL ENVIRONMENT

Molluscs

Intertidal bivalve molluscs have long been recognized for their ability to isolate themselves from air during low tide. Their well-developed mechanisms for facultative anaerobic metabolism enable them to remain isolated for many hours and even days (Dugal, 1939). It is now known that a variety of metabolic acids are produced anaerobically (de Zwaan, 1983). It is thought that the metabolic acids decrease the pH of fluids trapped within the shell from about 7.6 to as low as 6.6 (Dugal, 1939; Crenshaw and Neff, 1969; Crenshaw, 1972; Wijsman, 1975; Booth and Mangum, 1978; Jokumsen and Fyhn, 1982). However, Booth *et al.* (1984) have questioned this assumption, suggesting that the decline in pH is due to CO_2 produced aerobically from the small reserve of oxygen trapped within the shell. Regardless of the origin of the acidosis, Collip (1920, 1921) first suggested that calcium carbonate from the shell was used to buffer the acidosis. Crenshaw and Neff (1969) presented direct evidence that ^{45}Ca is taken up into the shell of the clam *Mercenaria mercenaria* and released again when the shells are tightly closed. The result of the shell buffering is an increase in both calcium ion concentration and total CO_2 content of the extrapallial fluid, the fluid between the mantle and the shell (Collip, 1920; Dugal, 1939; Crenshaw and Neff, 1969). The increase in Ca^{++} offsets the acidosis by increasing the strong ion difference (SID) (Stewart, 1981). However, since a closed bivalve represents a system closed with respect to CO_2, the accumulation of carbonate will lead to an increase in the P_{CO_2} of the fluid. P_{CO_2}, like SID, is an independent variable which determines the acid-base status of an aqueous solution. A rise in P_{CO_2}, therefore, acts to increase the H^+ concentration, thus offsetting the compensatory increase in SID. This paradoxical situation is addressed below along with other mechanisms of compensation.

Polychaetes

Among the polychaetes, the acid-base response of the lugworm, *Arenicola marina*, to emersion has been studied in detail (Toulmond, 1973). *A. marina* inhabits almost exclusively the intertidal zone where it lives in burrows in the sand. At low tide the burrow cannot be irrigated; *A. marina* remains isolated in its burrow where it rapidly depletes the oxygen reserves bound to hemoglobin. It is thought that the animal relies primarily on anaerobic metabolism during this time. Although both a metabolic and a respiratory acidosis occur during emersion, the acidosis is minimized by the presence of a fairly large Haldane effect, *i.e.*, as hemoglobin becomes deoxygenated it both becomes more alkaline and fixes CO_2 to form a carbamino compound. This is most obvious during the first hour of emersion when the blood P_{CO_2} increases slightly while pH rises due to the rapid deoxygenation of the hemoglobin. Thus, during a 4-hr period of emersion blood pH decreases from 7.42 to 7.35 (Toulmond, 1973), much smaller than the decrease to about 7.23 which would occur in the absence of a Haldane effect.

INTERACTION WITH THE AERIAL ENVIRONMENT

Decapod Crustaceans

Of all the intertidal organisms, the decapod crustaceans are probably the most active and the most thoroughly studied with respect to their blood acid-base balance upon emersion into the air. However, as I will point out, many important questions remain to be answered.

The decapod crustaceans are capable of making rapid transitions between the aquatic and the aerial environments. The behavioral patterns exhibited by the decapods upon emersion are highly varied. Many of the family Xanthidae, for example, remain somewhat inactive, hidden by clumps of oysters or burrowed in the mud. Others, such as the shore crabs of the family Grapsidae, are active both in and out of water and in some cases move readily from one medium to another regardless of the stage of the tide (Shoemaker and Burnett, 1983). Finally, the fiddler crabs (family: Ocypodidae) are more active in the intertidal zone when they are air exposed, becoming less active when immersed (unpublished observations).

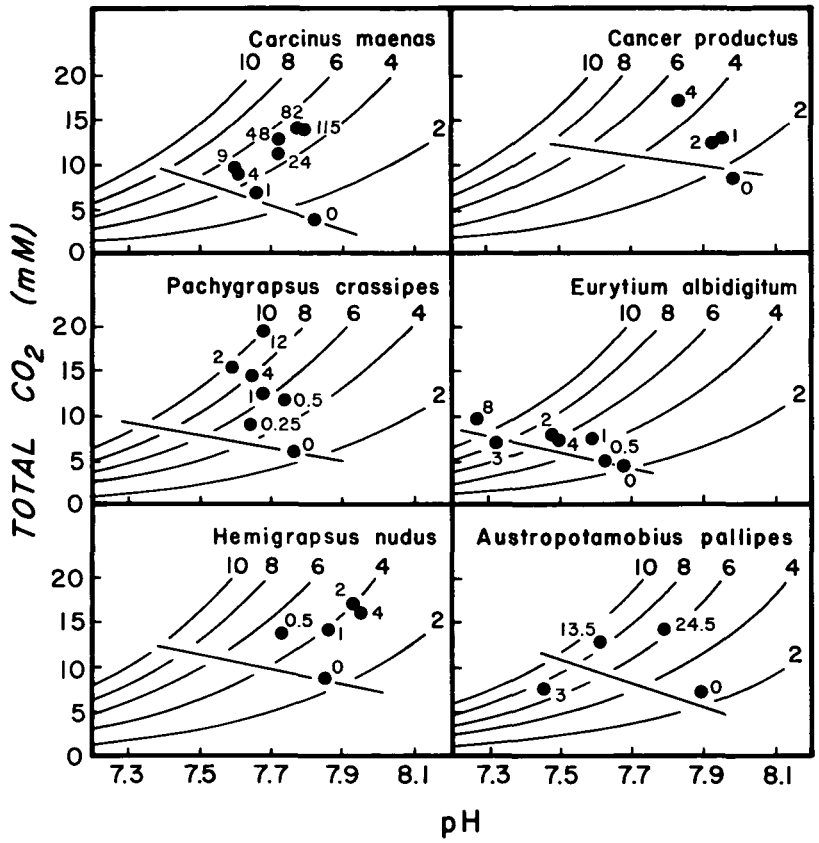

FIG. 1. pH-total CO_2 diagrams for different crustaceans showing the acid-base response to air exposure. P_{CO_2} isopleths shown at 2, 4, 6, 8, and 10 Torr are calculated using constants obtained by Truchot (1976). *In vitro* buffer lines are included with measured hemolymph variables indicated with the duration (hours) of air exposure (Truchot, 1975; Taylor and Wheatly, 1981; Wheatly and McMahon, 1982; deFur and McMahon, 1984*a*; Burnett and McMahon, 1987).

The decapod crustaceans by and large maintain aerobic metabolism during emersion although in some cases metabolism may be greatly reduced. This is due largely to respiratory adaptations of the gills to breathe air and/or modifications of the lining of the branchial chamber to exchange gases (deFur, 1988). In some cases these adaptations so favor an aerial mode of breathing that oxygen uptake in water is greatly reduced (O'Mahoney and Full, 1984).

The general response to air exposure is an increase in hemolymph P_{CO_2} which results in a respiratory acidosis (Fig. 1). The intertidal crabs *Cancer productus* (small ones), *Carcinus maenas, Eurytium albidigitum, Hemigrapsus nudus,* and *Pachygrapsus crassipes* as well as the freshwater crayfish *Austropotamobius pallipes* exhibit this response (Truchot, 1975; Taylor and Wheatly, 1980, 1981; deFur *et al.,* 1983; deFur and McMahon, 1984*b*; Burnett and McMahon, 1987). While *H. nudus* and *P. crassipes* compensate immediately for the ensuing acidosis, compensation in *C. maenas* and *A. pallipes* takes much longer (Turchot, 1975; Taylor and Wheatly, 1981). Compensation in *A. pallipes* is related in part to the disappearance of lactate from the hemolymph indicating that part of its metabolism is anaerobic (Taylor and Wheatly, 1981).

In *C. productus,* compensation is greater in small individuals (less than 100 g) compared with larger crabs (greater than 200 g) (deFur and McMahon, 1984*b*). The difference between the responses of the small

and large crabs is unknown, but may be due to the ability of the smaller crabs to better aspirate water into the branchial chambers (deFur and McMahon, 1984*a*). deFur *et al.* (1983) have examined the changes in hemolymph acid-base status of small *C. productus* in the intertidal zone *in situ,* and have postulated that the crabs are able to use the hypoxic interstitial water of the surrounding substratum as a pool for CO_2 excretion but not oxygen uptake. *E. albidigitum,* however, shows no compensation to the acidosis after 8 hr (Burnett and McMahon, 1987). The gills of *E. albidigitum* are imperfect gas exchangers in air as evidenced by a large decline in oxygen uptake (Burnett and McMahon, 1987). Thus, although CO_2 production must decline concomitantly with oxygen uptake, CO_2 is nonetheless retained.

Although there are no hemolymph P_{O_2} data available for *P. crassipes,* which compensates rapidly and nearly completely for the acidosis, the gills are obviously very effective at taking up oxygen from the air (Burnett and McMahon, 1987). The gills must also be relatively effective at excreting the large quantities of CO_2 generated when the crab is air exposed. Branchial carbonic anhydrase, shown to be present in *P. crassipes* (Burnett *et al.,* 1981), is involved in the CO_2 excretion process in air exposed crabs as has been shown for two other air breathing species, *Cardisoma carnifex* (Randall and Wood, 1981) and *Gecarcinus lateralis* (Henry and Cameron, 1983).

It is likely that mechanisms for maintaining hemolymph acid-base status in crabs contribute to their ability to maintain O_2 transport in air. The hemocyanins of *C. maenas* and *P. crassipes* both have moderate Bohr shifts (Truchot, 1971; Burnett and Infantino, 1984). A significant hemolymph acidosis, if uncompensated, would result in large pH reductions, greatly decreasing oxygen affinity (nearly doubling P_{50} in both cases). Therefore, compensation for the acidosis may be important in maintaining the integrity of the oxygen transport system during air exposure when hemolymph P_{O_2} declines (reviewed by deFur, 1988).

CO_2 exerts a specific, proton-independent effect on hemocyanin, increasing the oxygen affinity in *C. maenas* (Truchot, 1973; Mangum and Burnett, 1986), but not in *P. crassipes* (Burnett and Infantino, 1984), the only other intertidal decapod species where the phenomenon has been investigated. Thus, elevated hemolymph P_{CO_2} can offset the decrease in oxygen affinity due to the respiratory acidosis.

Morris *et al.* (1986) have shown that an increase in hemolymph lactate and Ca^{++} in *A. pallipes* during emersion serve to increase hemocyanin O_2 affinity. The presence of increased Ca^{++} in the hemolymph of crustaceans during air exposure (discussed below) may, in general, increase hemocyanin O_2 affinity, thereby facilitating O_2 uptake.

Limulus polyphemus

An interesting adaptation of the hemocyanin in the horseshoe crab, *Limulus polyphemus,* makes use of the respiratory acidosis during air exposure (Johansen and Petersen, 1975). During the mating season, *L. polyphemus* enters the intertidal zone where it is air exposed. Air exposure causes an increase in hemolymph P_{CO_2} from an average of 2.9 Torr to values ranging from 6.2 to 11.5 Torr. The integrity of the oxygen transport system during this time is maintained by the presence of a reversed Bohr shift (Johansen and Petersen, 1975; Mangum *et al.,* 1975). Thus, as hemolymph P_{CO_2} rises, the resulting acidosis causes a left shift in the oxygen equilibrium curve and oxygen uptake is maintained at about 36% of the immersed levels, with about 90% of the oxygen transported by the hemocyanin (Mangum *et al.,* 1975). It is interesting that the prosobranch mollusc *Busycon canaliculatum* also has a reversed Bohr shift (Mangum and Lykkeboe, 1979) which may be similarly adaptive when the large gastropod is air exposed at low tide.

MECHANISMS OF COMPENSATION

As Truchot (1983) has pointed out, the mechanisms of acid-base compensation in the decapod crustaceans are not known. However, according to Stewart (1981) in his quantitative treatment of acid-base bal-

ance, the only way a crab would have of bringing about the observed compensatory changes in hemolymph acid-base status would be to change any of the three independent variables which control acid-base status in an aqueous solution. These variables are the strong ion difference (SID), the total quantity of weak acid (A_{tot}) (or base), and the P_{CO_2}. Of the three variables, only P_{CO_2} has been measured routinely or calculated based on measurements of pH and total CO_2. The data indicate that during air exposure, P_{CO_2} rises and remains elevated for the duration, causing an acidosis. This leaves the manipulation of SID and A_{tot} as possibilities for compensation of the acidosis. Although neither of these two variables has been quantified in any invertebrate, there are strong indications that SID is the variable which is controlled, as I shall show below.

When *C. productus* is air exposed a respiratory acidosis occurs which is only partially compensated after 4 hr (deFur *et al.*, 1980). In the classical terminology, the respiratory acidosis is partially compensated by a metabolic alkalosis. The magnitude of the base excess calculated from the increase in hemolymph total CO_2 is about 2.2 m*M*. deFur *et al.* (1980) also measured a concomitant increase in hemolymph calcium ion concentration of about 9 m*M*. This change represents an increase in SID assuming the concentrations of all the other ions remain constant. Such an increase more than accounts for both the elevation of the total CO_2 concentration and the pH. Furthermore, since calcium is a divalent cation, each mmole of calcium contributes 2 mequivalents of charge to the SID. Thus, the actual increase in SID is probably about 18 mequivalents/liter. It has also been shown that hemolymph lactate concentration increases by about 8 m*M* (deFur and McMahon, 1984*b*; McMahon *et al.*, 1984). Since lactate is also a strong ion (Stewart, 1981), the increase will serve to lower the increase in SID brought about by calcium ions. deFur *et al.* (1980) and Henry *et al.* (1981) hypothesized that the source of the calcium is probably $CaCO_3$ from the shell. This is a perfect candidate for increasing the SID when branchial ion exchange mechanisms are cut off as they are during emersion, since calcium is a strong ion while carbonate is a weak electrolyte in the terminology of Stewart (1981). Thus, the concentration of carbonate in the solution depends on the values of the independent variables which determine acid-base status (P_{CO_2}, SID, and A_{tot}).

Recently, Cameron (1985) has demonstrated that calcium ions can indeed be released from the calcium carbonate stores within the shell. In this study, an acidosis was induced in the subtidal blue crab, *Callinectes sapidus*, by hypercapnia (high ambient P_{CO_2}). A hypercapnic acidosis, however, causes little or no change in hemolymph calcium concentration (Henry *et al.*, 1981; Cameron, 1985) and it would therefore be of interest to repeat Cameron's (1985) experiment on an air exposed intertidal species.

Again using *C. productus*, deFur *et al.* (1980) induced a respiratory acidosis in crabs immersed in hyperoxic water, which causes a hypoventilation of the branchial chambers. The acidosis was partially compensated after 24 hr resulting in a base excess of approximately 4 m*M*. This time, however, hemolymph calcium ion levels remained unchanged. The crab presumably used branchial ion exchange mechanisms to manipulate the SID. It must also be pointed out, however, that a significant discrepancy between the values of P_{CO_2} measured directly and the values of P_{CO_2} calculated from measurements of pH and total CO_2 was found in the deFur *et al.* (1980) study. The cause of the discrepancy and its meaning to the mechanisms of acid-base compensation are not understood.

Similar results were obtained by Henry *et al.* (1981) but using different experimental conditions. In this study the stimulus for the acidosis was hypercapnia. Using an aquatic and a terrestrial crab species held in aqueous and aerial environments, respectively, these authors were able to demonstrate two different kinds of compensation. Compensation for the hypercapnic acidosis in the terrestrial *Gecarcinus lateralis* is accompanied by an increase in hemolymph calcium ion levels (the electrical equivalent of about 9 mequivalents/

liter based on measurements of concentration), a result similar to that obtained in the air exposed *C. productus.* In the aquatic *C. sapidus,* compensation to the hypercapnic acidosis occurred with no increase in hemolymph calcium.

An increase in hemolymph calcium ion concentrations in response to an exercise-induced acidosis also occurs in the land crab *Cardisoma carnifex* (Wood and Randall, 1981*b*). The results from the three studies (deFur *et al.,* 1980; Henry *et al.,* 1981; Wood and Randall, 1981*b*) clearly indicate that crabs have the potential to manipulate SID in two fundamentally different ways which depend upon the availability of an aqueous medium. When an aqueous medium is unavailable, the crabs call upon the calcium carbonate stores of the carapace to buffer the acidosis. Otherwise, ionic exchange between the hemolymph and the aqueous medium at the gills accounts for the compensation.

In the bivalve molluscs, similar mechanisms of compensation are used. However, the origin of the acidosis in bivalves induced by air exposure is, in general, not clear. It was commonly assumed that the initial acidosis was due to accumulation of metabolic acids produced by anaerobic metabolism. In a study of the acid-base response to air exposure in the mussel *Mytilus edulis,* Booth *et al.* (1984) suggested that there was no metabolic acid component to the significant hemolymph acidosis which developed after 8 hr of air exposure. The acidosis occurred at the same time that tissues were producing metabolic acids (Walsh *et al.,* 1984). Booth *et al.* (1984) concluded that the acidosis was due to CO_2 produced aerobically from the limited oxygen stored within the shell. The metabolic acids produced anaerobically, therefore, remain in the tissues (Zurburg *et al.,* 1982). Booth *et al.* (1984) and Shick and Bayne (personal communication) further demonstrated that the acidosis induced by air exposure was partially compensated by the appearance of Ca^{++} and ammonium ions in the hemolymph.

As pointed out above, dissolution of the calcium carbonate shell plays a significant role in increasing the SID of the extrapallial fluid by increasing the calcium ion concentration. However, since the closed valves of the mollusc impose a closed system on the animal with respect to gas exchange, the ensuing increase in P_{CO_2} brought on by the carbonate from shell dissolution would tend to offset any advantage of the increase in SID. Fortunately, two factors occur which mitigate the increase in P_{CO_2}. Dugal (1939) and Crenshaw and Neff (1969) observed that the increase in calcium ion concentration in the extrapallial fluid was much greater on a molar basis than the increase in total CO_2. Crenshaw (1972) and Wijsman (1975) have solved much of this mystery by showing that, even when air exposed, the mussel *Mytilus edulis* opens its shells slightly during which time there is a concomitant rise in extrapallial fluid pH. This air gaping activity transforms the closed system to an open system (or at least partially open), allowing CO_2 to escape, preventing it from rising to very high levels. Air gaping also exposes the gills and mantle cavity tissues to O_2. Clearly, air gaping serves to both remove CO_2 and stimulate aerobic metabolism (Shick *et al.,* 1986, 1988).

Another factor may also offset the generation of CO_2 from shell dissolution. Since the extrapallial fluid compartment is separate from the sea water trapped within the shell, CO_2 generated in the extrapallial fluid compartment can easily pass into the sea water compartment without the concurrent movement of calcium ions. This activity would allow for significant shell buffering in molluscs which are buried in the substratum and therefore are unable to gape to expel CO_2, such as *Mercenaria mercenaria.* This may explain the curious jets of water which *M. mercenaria* sometimes propels into the air when intertidal mudflats are air exposed.

The precise manipulation of SID to control acid-base status is not well understood. There are no studies available which carefully document SID changes in invertebrates as a mechanism for acid-base balance control. Changes in SID mediated by changing the concentrations of the two most abundant ions, sodium and chloride, are the most likely candidates. Cameron

(1978), for example, has demonstrated that Na^+ influx in freshwater acclimated *C. sapidus* increases relative to Cl^- influx in response to ambient hypercapnia. This suggests that the hemolymph SID is increased by increasing the hemolymph sodium ion concentration to compensate for the induced acidosis. However, as pointed out above, the SID changes necessary to bring about compensation are on the order of several m*M*. Thus, minor changes in the concentrations of other strong ions may also account for compensation.

ROLE OF BRANCHIAL WATER STORES

In many intertidal organisms water is retained next to soft body tissues during the period of air exposure to prevent the tissues from drying out. In some cases, water retention keeps the respiratory surfaces moist and insures that gas exchange can continue during emersion. For example, the giant sea cradle *Cryptochiton stelleri,* although it prefers shaded crevices during emersion, curls the edge of its mantle upwards to expose its soft gills to the air (Petersen and Johansen, 1973). Although oxygen uptake in this chiton is reduced in air, it is able to maintain levels at roughly 30 to 45% of the immersed rate for 7 to 11 hr at a variety of temperatures. The importance of maintaining moist respiratory surfaces may be even more important in the small intertidal molluscs such as the limpets *Patella caerulea* and *Patella lusitanica* where oxygen uptake in air may be 50% and 300%, respectively, of that in water (Bannister, 1974).

Although the gills of the decapod crustaceans are covered with chitin, these surfaces are presumably also sensitive to desiccation (Grant and McDonald, 1979) and therefore must be kept moist. Water is retained in the branchial chambers of many different species (Carter, 1931; Gross, 1955; Flemister, 1958; Wheatly and Taylor, 1979; Wood and Randall, 1981*a*; Wolcott, 1984; Burnett and McMahon, 1987). Wood and Randall (1981*a*) suggest that water retention in *Cardisoma carnifex* may be associated with the presence of a spongy white pad along the inhalent margin over the legs. *C. carnifex* clearly uses its branchial water as a respiratory medium as reflected in the changes in branchial water P_{CO_2} and P_{O_2} in response to exercise. A decrease in P_{CO_2} and an increase in P_{O_2} occur in the branchial water as a result of an increase in ventilation at a time when CO_2 production and O_2 uptake increase. However, while all the mechanisms mentioned above serve to help maintain some level of oxygen uptake, the importance of water retention in relation to acid-base balance during emersion is not clear.

Recently, the role of branchial water stores in acid-base balance in the decapod crustaceans was investigated directly. Burnett and McMahon (1987) sampled the water in the branchial chambers of three species of crabs which were air exposed and found two different patterns of CO_2 accumulation. In the Grapsid crabs *P. crassipes* and *H. nudus,* total CO_2 in the branchial water increased rapidly during emersion, climbing from less than 2 m*M* to about 8 m*M* within 1 to 2 hr and remaining nearly constant for up to 12 and 4 hr respectively. The Xanthid crab *E. albidigitum,* on the other hand, accumulates no CO_2 in its branchial water for up to 4 hr and shows a significant, but small increase after 8 hr. Interestingly, the increase in total CO_2 in the branchial water of *P. crassipes* is correlated with the accumulation of a titratable base (Fig. 2). Although the nature of the base is unknown, it is most likely due to changes in the SID of the branchial water in this crab which regulates hemolymph Na^+ and Cl^-. In contrast, *E. albidigitum* adds little or no base to its branchial water (Fig. 2).

Burnett and McMahon (1987) suggested that the pattern of CO_2 and base accumulation in the branchial water was correlated with the ability of the crabs to osmoregulate. They further suggested that these observed patterns correlate with the ability of the crabs to compensate for an acidosis induced by air exposure. The evidence cited for this is that *H. nudus, P. crassipes, C. maenas,* and *A. pallipes,* all of which exhibit compensatory responses to the respiratory acidosis induced by air exposure, are good osmoregulators (Pros-

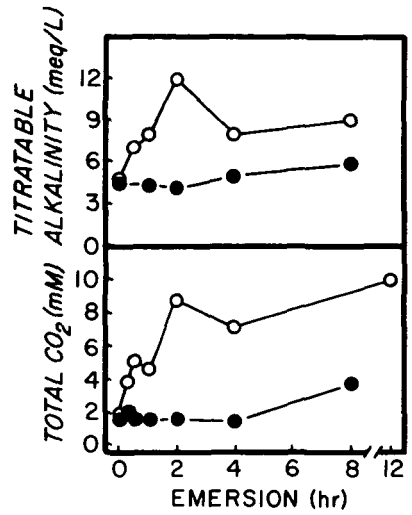

FIG. 2. Branchial water total CO_2 and titratable alkalinity in the crabs *Pachygrapsus crassipes* (open symbols) and *Eurytium albidigitum* (closed symbols) as a function of the duration of air exposure (Burnett and McMahon, 1987).

ser *et al.*, 1955; Shaw, 1961; Dehnel, 1962; Riegel, 1968). *E. albidigitum*, on the other hand, is an osmotic conformer and shows no compensation to the acidosis (Burnett and McMahon, 1987). These observations suggest that compensation, in part, depends upon some sort of interaction between the hemolymph and the residual water surrounding the gills. This interpretation lends support to the hypothesis that branchial ion exchange mechanisms are responsible for maintaining hemolymph acid-base status.

It is unclear, however, if base accumulation in branchial water represents an advantage for *P. crassipes* in removing CO_2 from the hemolymph. *P. crassipes* must produce more CO_2 than *E. albidigitum* during emersion (Burnett and McMahon, 1987). However, most of the trips *P. crassipes* makes into the air are of short duration, usually less than 10 min (Shoemaker and Burnett, 1983). With the rapid accumulation of base in the branchial water (Fig. 2) CO_2 would accumulate in the branchial water predominantly in the form of bicarbonate ions. This would serve to minimize the P_{CO_2} in the branchial water making conditions favorable for the movement of CO_2 out of the hemolymph. It is difficult to say what would happen in these conditions if base did not accumulate in the branchial water. Due to the large CO_2 production rate, perhaps P_{CO_2} in the branchial water would rise more rapidly, reducing the gradient for CO_2 excretion, and a larger respiratory acidosis would occur.

In two studies of the mechanisms of compensation to acid-base disturbances, Truchot (1975, 1979) detected a net base excretion (or acid uptake) in the post reimmersion period of air exposed *C. maenas*. Other lines of evidence led him to conclude that the gills were the major site of base excretion and that urinary excretion is of minor importance to base excretion. These results are qualitatively similar to the ones obtained by Burnett and McMahon (1987). Furthermore, Truchot (1979) found a discrepancy in the quantity of base eliminated from the hemolymph and the quantity of base appearing in the branchial water in the post reimmersion period leading him to conclude that exchange processes include not only the hemolymph compartment and the external medium (branchial water) but also other compartments.

As suggested above, intertidal crabs probably use calcium carbonate derived from the shell to compensate for the respiratory acidosis which occurs during air exposure. Base (*e.g.*, Na^+) excreted from the hemolymph compartment into the branchial water stores would have little impact on the quantity of base in the hemolymph, since the sizes of the branchial water stores are small in the two species where its size has been measured. Consider *P. crassipes* weighing 20 g. If the size of the hemolymph compartment is 6 ml (30% of body mass based on Gleeson and Zubkoff, 1977) and the size of the branchial water stores is 0.26 ml (Burnett and McMahon, 1987), then an increase in the titratable alkalinity of the branchial water by 5 mequivalents/liter (Burnett and McMahon, 1987) would decrease the amount of titratable base in the hemolymph compartment by only 0.2 mequivalents/liter. This depletion of base from within the hemolymph compartment is an amount which can eas-

ily be made up with the addition of calcium from the exoskeleton. Thus, the appearance of base in both the hemolymph and the branchial water during air exposure is accounted for by the much smaller volume of the branchial water stores compared with that of the hemolymph.

CONCLUSIONS AND FUTURE RESEARCH

The options available to an organism which lives within the intertidal zone appear to be few. Organisms which are closely tied to their habitat either because of their soft bodies (as in the case of polychaetes) or their relative lack of mobility (as in the case of bivalve molluscs) must obviously tolerate the lack of access to an outside medium for purposes of feeding and gas exchange. Confronted with a relative lack of oxygen these organisms switch to a predominantly aerobic mode of metabolism. An acidosis resulting from the production of metabolic acids during this period clearly is minimized but not completely compensated.

On the other hand, organisms which are free to actively move and explore different habitats during air exposure by and large maintain some level of oxygen uptake from the air, although perhaps at greatly reduced rates. An acidosis in the decapod crustaceans, the best studied group, is due to the retention of metabolically produced CO_2. Compensation for the acidosis may be nearly complete or not at all. The mechanisms of compensation appear to be similar to that observed in the molluscs, *i.e.*, the dissolution of $CaCO_3$ stores to produce free calcium ions. However, unlike the molluscs it is much easier for the crustaceans to remove excess CO_2 generated by the carbonate ions. Finally, there is a suggestion that branchial water stores may also be important in the process of compensation over the short term by trapping CO_2 as bicarbonate and thus maximizing the CO_2 gradient between the hemolymph and the branchial water.

There are several different areas which are important for future investigations. The picture of acid-base balance in the bivalve molluscs is not at all clear. Monitoring the changes in the acid-base status of extrapallial fluid and trapped sea water in the closed bivalves would clarify the role of calcium ions from shell dissolution in compensating for the metabolic acidosis and the fate of CO_2 generated by this process. These fluids may also be suitable for titration and thus direct, empirical determination of SID (Stewart, 1981).

While the picture of acid-base balance in the decapod crustaceans during air exposure is fairly clear, knowledge of the specific ionic changes which occur to bring about compensation would be very instructive in understanding general mechanisms of compensation in response to other stimuli such as hypoxia, exercise, and perhaps acid rain. The correlation between the ability to osmoregulate and the ability to compensate for an air exposure induced acidosis provides an interesting hypothesis which should be tested on other crustaceans which show a range of osmoregulatory responses.

Finally, the role of branchial water stores in maintaining acid-base balance during air exposure needs further investigation in the decapod crustaceans. But this is also fertile ground for studies on other groups where substantial water stores are retained during emersion.

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