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TRANSEPITHELIAL MOVEMENT OF CALCIUM IN CRUSTACEANS

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Summary

The regulation of calcium in most crustaceans is especially challenging owing to the highly mineralized cuticle that must be recalcified after each moult, a process that often occurs in environments with low concentrations of calcium. The gill and carapace epithelia separate the major calcium-containing compartments of the body and therefore see large changes in the rate of calcium flux through the moult cycle. Large changes in the ultrastructure of these cells do not, however, correlate well with the periods of calcium movement and probably reflect other physiological events. Despite the challenges to regulating calcium levels at various acclimation salinities and moult stages, the calcium concentration in the blood is maintained relatively constant. There is a rapid increase to a high rate of calcium flux across both the gill and carapace epithelium shortly after the moult; on an area-specific basis these fluxes are among the highest reported for calcium-transporting epithelia. When in water with a very low concentration of calcium, the electrochemical gradient for calcium is directed outwards and net influx must occur by active transport. Evidence suggests that changes in the electrochemical gradient, permeability and active transport are all important in the ability of crustaceans to take up calcium from water with a low concentration of this ion. Although an enzyme transporter is presumably involved in the active transport of calcium across epithelia, very little is known about the cellular mechanism of the transepithelial movement of calcium in crustaceans.

Introduction

Owing to its chemical characteristics, the calcium ion is not only a regulatory agent in physiological processes but also the primary cation used in biomineralized structures. Maintenance of an extremely low intracellular concentration of calcium is particularly problematic in those cases where there are large calcium fluxes across tissue layers resulting from changes in the overall calcium balance of an organism. Organisms such as crustaceans that have highly calcified structures thus present particular challenges for the maintenance of proper intra- and extracellular concentrations of calcium; relatively large quantities of calcium must be moved across epithelial layers and through the

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circulatory system. The accretionary growth of crustaceans entails discarding the old cuticle, normally representing the loss of a massive quantity of calcium. In most crustaceans, a large quantity of calcium must subsequently be replaced in a relatively short period to regain the structural and protective functions of the mineralized cuticle. In addition to massive fluxes of calcium into the cuticle after the moult, in some cases this is preceded by a period of calcium transport in the opposite direction as the cuticle is partially demineralized (Sparkes and Greenaway, 1984; Wheatly and Ignaszewski, 1990).

Crustaceans employ a diversity of strategies allowing them to mineralize the cuticle while occupying habitats with a wide range of calcium availabilities. They inhabit environments ranging from hypersaline, where there is a relative abundance of calcium, to freshwater or terrestrial environments, where acquiring sufficient amounts of calcium is more difficult (Mantel and Farmer, 1983). Even for those species living in fresh water, calcium concentrations vary greatly. Some crayfishes inhabit water with calcium concentrations of less than $100\,\mu mol\,l^{-1}$ (e.g. Malley, 1980), whereas euryhaline crabs such as *Callinectes sapidus* typically inhabit fresh water with a calcium concentration of around $1 mmol\,l^{-1}$ (Cameron, 1978). Species living in water with low concentrations of calcium often reduce the need to acquire large amounts of calcium from the environment by shifting it to the blood or to organs of the gastrointestinal tract prior to moulting. Marine crustaceans, in contrast, tend to rely more on the availability of calcium in the external milieu by taking up large quantities of calcium *via* the gill. The prominent role of calcium in the physiology of crustaceans, coupled with the diversity of habitats occupied by these organisms, therefore presents unique examples of calcium regulation at both the organismic and cellular levels.

While there is little information on the mechanism of calcium transport in crustaceans, changes in the ultrastructure and physiology of calcium-transporting tissues have given indications of factors that are important for calcium regulation. This article will review ultrastructural and physiological data relevant to the transport of calcium across the primary epithelia involved in the regulation of this cation in crustaceans. Particular emphasis will be given to our recent studies in the euryhaline blue crab, *Callinectes sapidus*, a good model for calcium regulation in a crustacean since it ranges across a variety of salinities (Gifford, 1962).

Ultrastructure of epithelia involved in calcium transport

By virtue of their location as barriers between the primary calcium-containing compartments of the body, the gill and carapace epithelia are intimately involved in the regulation of calcium levels in crustaceans and provide the most information with respect to calcium transport. The gills function as the primary interface between the external medium and the blood, while movements of calcium between the blood and carapace occur across the epithelial layer underlying the carapace. The various epithelia of the gastrointestinal tract are similarly located to see changing fluxes of calcium resulting from dietary or calcium storage functions, but there is less known about these epithelia so we will concentrate on the gill and carapace epithelia.

Gill epithelium

The epithelium lining the gill is the tissue that has been most extensively studied with regard to ionoregulatory processes in aquatic crustaceans. The gill can be partitioned into a thin (<1 μm) epithelium, presumed to function primarily in respiration, and a thick (approximately 10 μm) epithelium, presumed to function mainly in ionoregulation (Copeland and Fitzjarrell, 1968). While the thin epithelium contains few organelles and little cellular elaboration, the thicker epithelium has many of the characteristics of other transporting epithelia: an abundance of mitochondria, a large cell surface area and well-defined cell junctions (Berridge and Oschman, 1972). Ultrastructural evidence for the role of the thick epithelium in ionoregulation comes from the large structural changes observed during salinity acclimation (e.g. Compere *et al.* 1989). Functionally, the localization of Na^+/K^+-ATPase activity in cells of the thick epithelium suggests that these cells are the site of ionoregulatory processes (Neufeld *et al.* 1980); by extension, these same cells are considered to be the most likely candidate for the site of calcium regulation.

While there are changes in gill ultrastructure with salinity acclimation, there is little indication of which morphological changes might be involved in calcium regulation as opposed to the regulation of other ions. The physiological events at the moult are better suited for a correlation of calcium transport with cell structure, since the period of maximum flux occurs at a well-defined period and can be temporally separated from other physiological changes. Unfortunately, there is very little information on what ultrastructural changes occur in the gill through the moult cycle. Unlike Andrews and Dillaman (1993), who report no change in the epithelium of *Procambarus clarkii* through the moult cycle, we observed significant changes in epithelial height and mitochondrial abundance in the gill epithelium of *Callinectes sapidus* acclimated to low salinity. The increased cell activity occurred during the premoult period, however, correlating with the period when organic materials are deposited onto the new cuticle rather than the period of calcium deposition (D. S. Neufeld and J. N. Cameron, unpublished observations). Aside from the presence of multivesicular bodies, the gill epithelium at postmoult had the same appearance as that at intermoult. These results suggest that the metabolic expenditure by the gills for calcium transport is similar to the metabolic expenditure during the intermoult period, even when *Callinectes sapidus* moults in low salinities where calcium uptake must occur by active transport (Neufeld and Cameron, 1992).

Carapace epithelium

Regardless of whether calcium for mineralization comes from the external medium or from stored reserves, calcium deposited onto the cuticle must move across the epithelial layer that forms a barrier between the cuticular space and the blood. In the various crustaceans studied, substantial changes in ultrastructure are observed through the moult period (reviewed by Roer and Dillaman, 1984). In *Callinectes sapidus* this layer is quite thin during intermoult, having very few organelles and little cell surface elaboration. As with the gill epithelium, we observed an enlargement of the carapace epithelium far in advance of the moult and this enlargement was concomitant with the appearance of more

mitochondria, endoplasmic reticulum and vesicles. Hypertrophy of the epithelium at this stage is expected, given that synthesis of the organic portion of the carapace begins early in the premoult stage. It is surprising, however, that the carapace epithelium decreases greatly in both size and complexity shortly after moult when large quantities of calcium are being deposited into the new carapace. The general regression of the carapace epithelium after the moult suggests that the movement of calcium requires little cellular effort in comparison with other physiological functions of the epithelium during the moult period, as is the case in the gill epithelia.

Calcium concentrations in the blood

As the main calcium-transporting tissues, the gill and carapace epithelia are the primary epithelia responsible for the maintenance of appropriate calcium concentrations in the blood and cuticle. Changes in the calcium concentration of the blood are therefore due either to differences in flux rates across these tissues or to changes in the size of the blood compartment. Many more studies have described the pattern of calcium regulation in the blood in response to the two primary physiological challenges for calcium regulation, salinity acclimation and moulting (see review by Greenaway, 1985), than have described the actual mechanisms responsible for this pattern. Despite the challenges placed on crustaceans by moulting and the diversity of strategies used to deal with the large requirement for calcium, calcium concentration in the blood is generally maintained within a narrower range than that of other ions, no doubt because of its numerous regulatory functions.

Salinity

The concentration of total calcium in the blood is relatively constant not only within a species acclimated to various salinities but also between species which normally inhabit a variety of salinities. The concentration of total calcium in the blood is most commonly between 10 and 20mmol l^{-1} (Mantel and Farmer, 1983). Since calcium movements are thermodynamic processes, however, the concentration of free calcium is actually the more relevant variable. Free calcium was unfortunately more difficult to measure until the recent advent of calcium-selective electrodes, which now allow the direct measurement of that portion of the total calcium not bound to proteins or anions. There are not enough data currently available to determine whether a correlation exists between habitat and the quantity of calcium in bound *versus* free form. The marine species *Callinectes sapidus* (Neufeld and Cameron, 1992) and *Carcinus maenas* (Greenaway, 1976) have approximately 30% of calcium in the bound form. While the freshwater crustaceans *Gammarus pulex* (Wright, 1979) and *Austropotamobius pallipes* (Greenaway, 1972) have a larger percentage (50–60%) of the total calcium in a bound form, the freshwater *Holthuisana transversa* has only 20% of calcium in the bound form at intermoult (Sparkes and Greenaway, 1984). Within a single species, the concentrations of free and total calcium in the blood of *Callinectes sapidus* are independent of the acclimation salinity (Fig. 1), indicating that, for this euryhaline crab, the regulation of calcium relies on compensatory mechanisms rather than on a shift in the concentration of

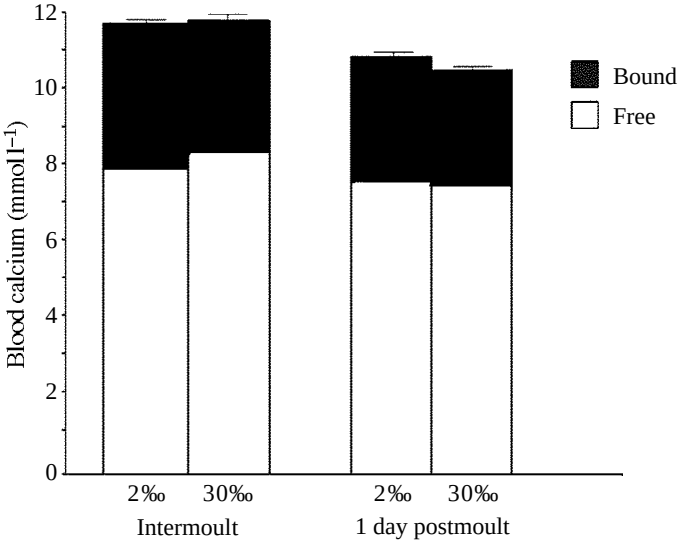

Fig. 1. Distribution of blood calcium in *Callinectes sapidus* demonstrating little change in total or free calcium with moult stage or acclimation salinity(2‰ or 30‰). Top error bars are for measurements of total calcium; lower error bars are for measurements of free calcium (from Neufeld and Cameron, 1992).

calcium maintained in the blood. There are no other studies indicating whether this is a general feature in euryhaline crustaceans, but it appears that total and free calcium may be equally independent of acclimation salinity.

Moulting

While salinity appears to have little effect on the calcium concentration in the blood, the regulatory mechanism for calcium is obviously presented with an added challenge during the moult cycle of crustaceans, when there are large fluxes of calcium. Among various species, the concentration of total calcium consistently shows an increase during the period prior to the moult (Greenaway, 1985). At least in *Callinectes sapidus*, this is most likely to be due to changes in blood protein and concomitant changes in the amount of bound calcium rather than to a change in the concentration of free calcium (Neufeld and Cameron, 1992). The most extreme example of a premoult rise in blood calcium is found in terrestrial/freshwater species of crabs such as *Holthuisiana transversa,* where the total concentration of calcium can increase 150-fold to a concentration of over $2\,mol\,l^{-1}$ by the formation of calcite spherules, accounting for approximately 30% of the blood volume (Sparkes and Greenaway, 1984). The premoult increase in calcium concentration in most species is followed by a decrease at ecdysis, presumably due to dilution of the blood by the large quantity of water taken up as the body swells. Although the decrease in total calcium at moult in the blood of most species is relatively small, blood calcium level drops substantially in some species that take up water containing very low concentrations of calcium (Wright, 1980).

Data on the relationship between the concentration of free calcium and moult stage are much more scarce. Free calcium in the blood of *Callinectes sapidus* acclimated to low salinity changes little from intermoult to postmoult (Fig. 1), a pattern of stasis also found in the freshwater crayfish *Austropotamobius pallipes* (Greenaway, 1974*a*,*b*). The greatest change reported is for *Holthuisana transversa*, where calcium activity decreases by 25% at moulting (Sparkes and Greenaway, 1984). In other cases, the drop in total calcium is great enough that free calcium must drop by a substantial degree as well (Wright, 1980). Unlike Towle and Mangum (1985), we did not find a substantially lower concentration of free calcium after the moult in *Callinectes sapidus* acclimated to sea water (Fig. 1). Given the large dilution of the blood as water is absorbed for expansion of the body, it is somewhat surprising that large decreases in total or free calcium are not seen more frequently. This probably indicates a transport mechanism well suited to the rapid recovery of a steady calcium concentration in the blood. Although the changes in free calcium are relatively small, a drop in the calcium activity after moulting would serve to create a more favourable electrochemical gradient for calcium uptake after the moult. In the case of *Callinectes sapidus*, where there is a slight drop in both free (Towle and Mangum, 1985) and total calcium (Cameron, 1989), the electrochemical gradient for calcium is directed inwards and net calcium influx may occur by passive mechanisms (Cameron, 1989). Even in crustaceans inhabiting water with lower concentrations of calcium, where the electrochemical gradient is directed outwards and uptake must occur by active transport, a lower calcium concentration in the blood after the moult may serve to reduce passive losses or to lessen the electrochemical gradient against which calcium is transported.

Flux rates of calcium

Intermoult

There are very few reports of the rate of unidirectional influx for crustaceans during the intermoult period. Those rates reported suggest that unidirectional influx is low at intermoult, at least in comparison with the massive fluxes at postmoult (Fig. 2). The freshwater crayfish *Austropotamobius pallipes*, *Gammarus pulex* and *Orconectes virilis* have influx rates of 0.014 (Greenaway, 1972), 0.287 (Wright, 1979) and $0.77 mmol kg^{-1} h^{-1}$ (Malley, 1980), respectively. The only influx rate measured for a marine crustacean, *Carcinus maenas*, is $0.5 mmol kg^{-1} h^{-1}$ (Greenaway, 1976). While there is some exchange of calcium between the blood and carapace at intermoult (Dall, 1965; Greenaway, 1976; Roer, 1980; Henry and Kormanik, 1985), exchange across this layer is certainly relatively low in comparison with postmoult fluxes.

Premoult

During intermoult, there is either no net flux (Greenaway, 1976; Wheatly and Ignaszewski, 1990; Neufeld and Cameron, 1992) or only a small loss (Greenaway, 1972; Wright, 1979) of calcium, but some species lose a substantial amount of calcium immediately prior to the moult as the old cuticle is partially dissolved. Roer (1980) measured flux rates in isolated carapace tissues during the premoult period and found the flux of calcium from the cuticle to be greater than the flux in the opposite direction. When

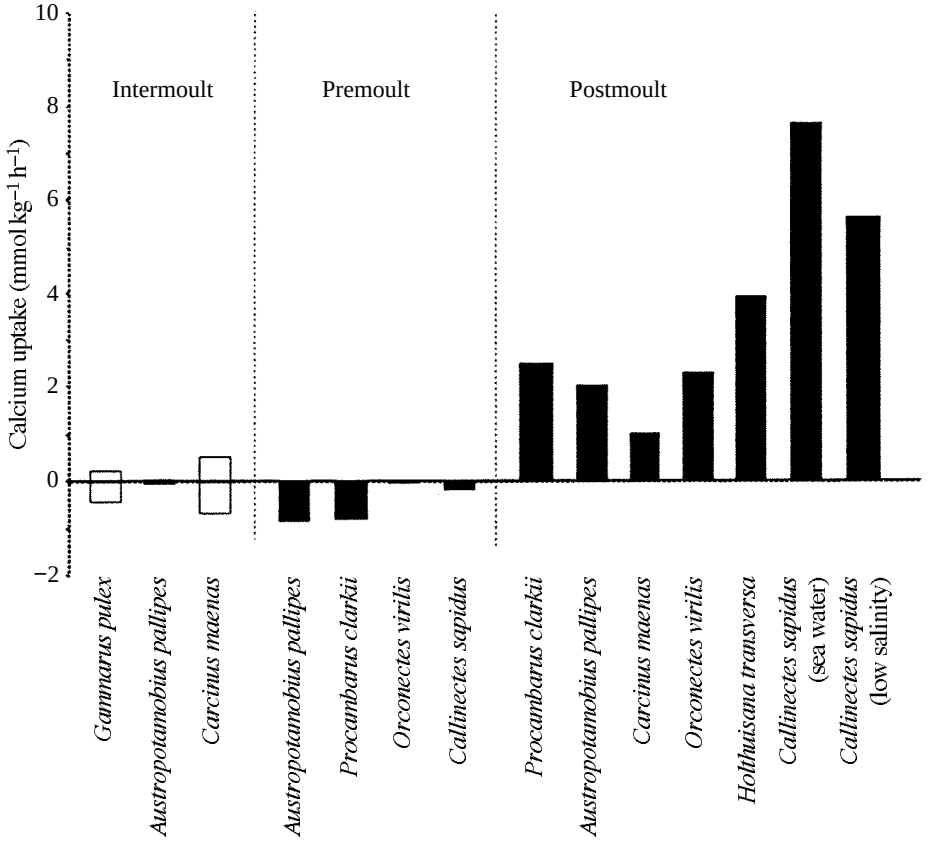

Fig. 2. Rate of calcium uptake in crustaceans at various moult stages. Filled bars represent net uptake of calcium, open bars represent unidirectional fluxes of calcium. (Data are replotted from Greenaway, 1972, 1974*a*,*b*, 1983; Wright, 1979; Malley, 1980; Sparkes and Greenaway, 1984; Cameron and Wood, 1985; Wheatly and Ignaszewski, 1990; Neufeld and Cameron, 1992).

detectable, the magnitude of the calcium flux from the cuticle at premoult is generally not comparable to the large postmoult influx but it can represent a significant change from intermoult fluxes (Fig. 2). In still other cases, movement of calcium onto the new cuticle begins prior to the moult (Henry and Kormanik, 1985; Wheatly and Ignaszewski, 1990).

Postmoult

There are more reports of flux rates for the postmoult period when the net flux onto the new carapace is very high. The measurement of net fluxes across the gill is facilitated by the good correlation between apparent hydrogen excretion and net calcium uptake (Cameron, 1985; Wheatly and Ignaszewski, 1990). Calcification of the cuticle occurs by the reaction $Ca^{2+} + HCO_3^- \rightarrow CaCO_3 + H^+$, in which bicarbonate is provided either directly from the external solution or by hydration of metabolic CO_2, which results in the

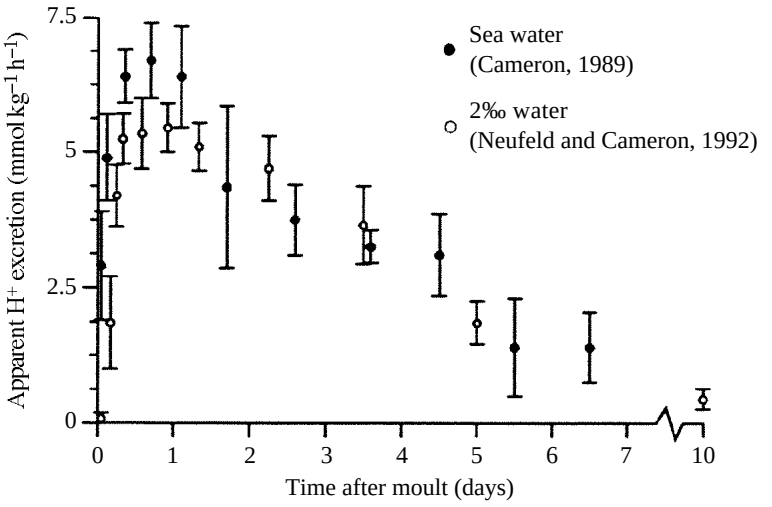

Fig. 3. Comparison of the rate of apparent hydrogen ion excretion (directly proportional to calcium uptake) after the moult of *Callinectes sapidus* at two acclimation salinities. Values are mean ± S.E.M., N=3–11.

production of an extra hydrogen ion for excretion. The effect on external pH is the same regardless of the source of bicarbonate, allowing the change in external pH to be used as an indirect measure of calcium uptake. Flux rates given for the postmoult period in Fig. 3 are from a variety of salinities, temperatures and organism sizes, limiting the validity of either inter- or intraspecific comparisons. Nonetheless, the rate of net uptake is similar for animals under a variety of conditions, ranging from 1 (Greenaway, 1983) to 7.5mmol$kg^{-1}h^{-1}$ (Cameron and Wood, 1985). While a reduction in net influx of calcium might be expected in calcium-limited environments, the evidence suggests instead that freshwater species are able to accumulate calcium as rapidly as their marine counterparts. We found, for instance, the time course and magnitude of calcium uptake in *Callinectes sapidus* to be identical in low-salinity water and sea water (Fig. 3).

The rate of net flux gives a lower boundary for the rate of unidirectional influx occurring across the gill, since the body is permeable to calcium. Crustaceans living in environments where the electrochemical gradient is directed outwards at the time of calcium uptake are especially apt to lose a proportion of the calcium taken up, since there is a general increase in permeability to water and ions after the moult (Lockwood and Andrews, 1969). The only unidirectional efflux at postmoult reported is for *Orconectes virilis*, where the efflux rate is 0.014mmol$kg^{-1}h^{-1}$, several orders of magnitude lower than the influx rate at this stage (Malley, 1980). It seems likely, therefore, that unidirectional influx at postmoult is only slightly higher than the net fluxes usually measured.

Comparison of flux rates in various tissues

Using mass-specific rates of calcium movement into the body and estimates of gill

areas, flux rates can be calculated on an area-specific basis for comparison with flux rates in other calcium-transporting tissues. In *Callinectes sapidus*, we calculated the average rate of calcium uptake across the gills to be approximately 3000nmol$cm^{-2}\,h^{-1}$ at the time of maximal calcium uptake. Ultrastructural evidence discussed earlier points to functional differences in areas of the gill, making it likely that some areas of the gill actually see much greater flux rates. These flux rates are in fact among the highest reported for calcium-transporting tissues (Table 1), indicating that crustaceans have an impressive ability to transport calcium in comparison with other tissues. Estimating flux rates across the carapace epithelium is somewhat more problematic, since direct measurement of cuticular area is difficult. Various attempts to measure fluxes in isolated carapace epithelia (Roer, 1980; Henry and Kormanik, 1985) have reported rates much lower than those estimated by the rate of appearance of calcium in the cuticle (Vigh and Dendinger, 1982). The rate of deposition into the cuticle of *Holthuisana transversa* (50mmol$kg^{-1}\,h^{-1}$) is an order of magnitude higher than flux rates for calcium across the gills of crustaceans (Sparkes and Greenaway, 1984); the area-specific fluxes in this species may rival the high flux rate in the avian oviduct (Table 1).

Dependence on external salinity or calcium concentration

Response to abrupt changes

The rate of calcium uptake at various external concentrations is roughly described by saturation kinetics in a manner similar to the uptake of other ions (Mantel and Farmer,

Table 1. *Net rate of Ca^{2+} uptake across various transporting epithelia*

Species	Epithelia	Flux rate ($nmol\,cm^{-2}\,h^{-1}$)	Source
Rat	Duodenum	48–88	Favus *et al.* (1983)[1]
Rat	Colon	11–17	Favus *et al.* (1983)[1]
Rat	Kidney	192–328	Costanzo and Windhager (1978)[2]
Hen	Oviduct	20000	Romanoff and Romanoff (1949)[3]
Tilapia	Opercular membrane	0.31–0.76	McCormick *et al.* (1992)[4]
Trout	Opercular membrane	0.036–0.043	Marshall *et al.* (1992)[5]
Oyster	Mantle epithelium	175	Wilbur and Jodrey (1952)[6]
Snail	Uterus	3000	Wilbur and Tompa (1979)[3]
Prawn	Intestine	140	Ahearn (1980)[7]
Crab	Gill	3000	Cameron and Wood (1985); Neufeld and Cameron (1992)[8]

[1]*In vitro*; [2]*in situ*; distal tubule; [3]cited in Wilbur (1980); [4]*in vitro*; tilapia in fresh water and low-Ca^{2+} fresh water; [5]*in vitro*; trout in fresh water; [6]calculated on the basis of the amount of ^{45}Ca incorporated in the shell; oysters in sea water; [7]*in vitro*; prawns in fresh water; [8]calculated on the basis of the total gill area ($162\,cm^2$).

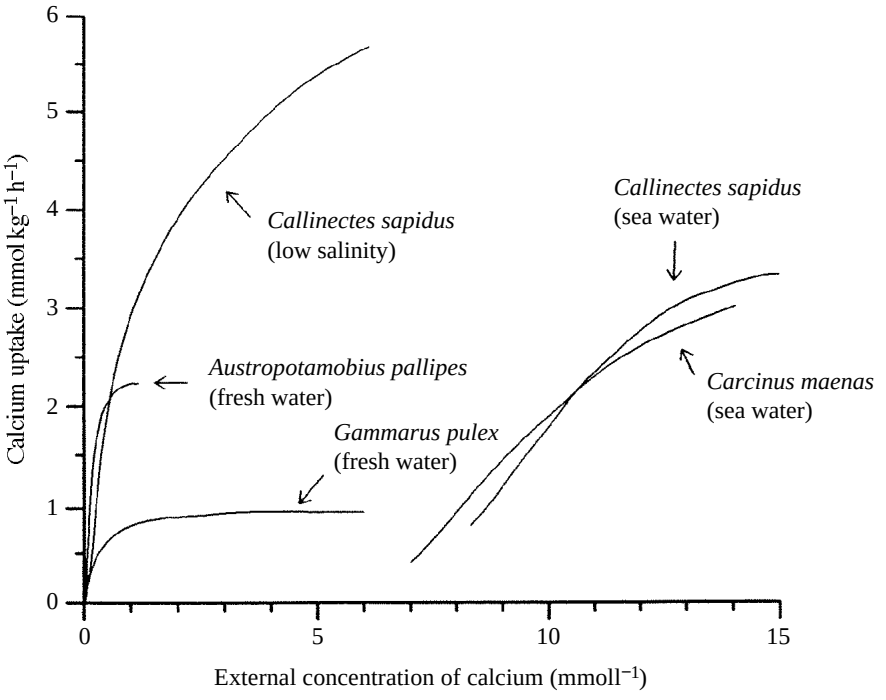

Fig. 4. Dependence of calcium uptake on the external concentration of calcium in crustaceans acclimated to various salinities (curves redrawn from Greenaway, 1974*b*, 1983; Neufeld and Cameron, 1993; Wright, 1979). The curve for *Gammarus pulex* represents unidirectional influx of calcium at intermoult, all other curves represent net uptake of calcium at postmoult.

1983) and has been used to define an affinity and maximum transport rate of the calcium transport system of various species. As would be expected, organisms inhabiting low salinities have systems with higher affinities (Fig. 4). In *Callinectes sapidus* acclimated to low salinity, we attempted to determine the basis for the response of calcium uptake to various external concentrations of calcium by correlating the electrochemical gradient for calcium with the flux rate for calcium (Neufeld and Cameron, 1993). The dependence of calcium uptake on external calcium was positively correlated with the direction and magnitude of the electrochemical gradient (Fig. 5). In water with lowered concentrations of calcium, the transepithelial potential shifted slightly to become more negative and free calcium in the blood decreased. These adjustments were not sufficient to offset a much lower concentration of calcium in the external solution, however, and the electrochemical gradient became more strongly directed outwards. In the absence of information about the mechanism of calcium transport across the epithelium, it is difficult to assess how a change in the electrochemical gradient would exert an effect on flux rates. Given the extremely low intracellular activity of calcium and the negative potential of the cytoplasm relative to external fluids, calcium movement across the apical surface of the cell may be down a strong electrochemical gradient and the subsequent movement across the basolateral surface may be against a strong electrochemical gradient, regardless of the

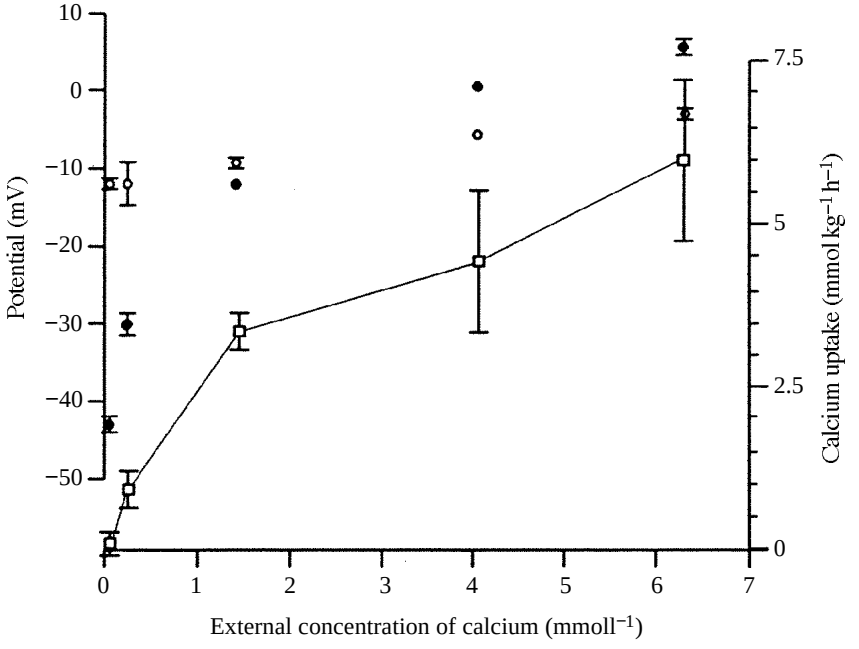

Fig. 5. Response of the net uptake of calcium (□), the transepithelial potential (○) and the equilibrium potential (●) for calcium on the external concentration of calcium, demonstrating the correlation of calcium uptake with the electrochemical gradient for calcium (data from Neufeld and Cameron, 1993). Values are mean ± S.E.M., *N*=8–32.

electrochemical gradient across the epithelium as a whole. The overall electrochemical gradient for calcium would still be important for net fluxes, however, since passive paracellular flux rates are dependent on the electrochemical forces. The difference in regulatory abilities at various external concentrations of calcium could, therefore, be the result of changes in passive fluxes rather than any difference in active uptake.

Long-term acclimation

While an uptake curve that saturates at low calcium concentrations could reflect the presence of an active transport mechanism with a higher affinity in crustaceans living in lower salinities (Fig. 4), uptake curves for various species were measured at different salinities and some of the differences are probably due to the effect of changes in electrical and chemical forces acting on passive fluxes or to changes in permeability. It is doubtful whether such differences are due solely to changes in the electrochemical gradient, however; acclimation probably involves a direct change in the calcium transport system. In *Callinectes sapidus*, we found that postmoult animals acclimated to sea water showed a drop in net calcium uptake when moved to low salinity. This change could not be accounted for by the electrochemical variables but was evidently due to a change in the active transport component or to a change in permeability (Neufeld and Cameron, 1993). *Callinectes sapidus* has a relatively high permeability for sodium and chloride (Cameron,

1978); a similarly high calcium permeability may prevent its excursion into waters with lower calcium concentrations. One of the few ultrastructural changes that we observed when *Callinectes sapidus* was acclimated to low salinity was a notable increase in the length of the cell junctions, perhaps causing a change in ionic permeability. Further support for the importance of permeability in acclimation stems from direct studies of *Carcinus maenas*, where a drop in salinity did not cause an increase in the rate of calcium efflux despite a stronger electrochemical gradient directed outwards, indicating a decrease in permeability of the epithelium (Greenaway, 1976). Unidirectional efflux of calcium is initially high when intermoult *Austropotamobius pallipes* is transferred to low-calcium water, despite a negative shift in the transepithelial potential (Greenaway, 1972). A subsequent decrease in calcium loss along with a depolarization of the transepithelial potential suggests that decreased permeability also plays a role in acclimation in this freshwater species. A general reduction in permeability is one of the general methods of acclimation with respect to osmo- and ionoregulation in crustaceans (Mantel and Farmer, 1983).

Active transport

The studies mentioned previously suggest a role for permeability and electrochemical forces in the regulation of calcium. As an alternative mechanism of acclimation, calcium regulation may rely on compensatory mechanisms. In the case of crustaceans that inhabit low salinities or fresh water, assessment of the electrochemical gradient for calcium implicates active transport across the gill epithelium. In *Callinectes sapidus* acclimated to low salinity, we found the equilibrium potential for calcium to be more negative than the transepithelial potential at all moult stages, indicating that active transport is responsible for the calcium movements (Neufeld and Cameron, 1992). Active transport is similarly required for calcium uptake in *Austropotamobius pallipes* (Greenaway, 1974*b*) and *Gammarus pulex* (Wright, 1979) when in water with low concentrations of calcium. The electrochemical gradients for calcium in *Austropotamobius pallipes* and *Callinectes sapidus* are similar at intermoult and postmoult, since there is little change in the chemical or electrical gradients for calcium (Greenaway, 1974*b*; Neufeld and Cameron, 1992). The large increase in net uptake of calcium is therefore due to an increase in the influx component.

In mammalian models, where there is much more information on the mechanism of calcium transport, there is still disagreement about the details of calcium movement at the cellular level (Bawden, 1989). The most plausible mechanism involves apical entry into the cell down the steep electrochemical gradient created by a low internal calcium concentration and a negative potential. Calcium may cross the cell *via* intracellular shuttles that prevent drastic elevations in intracellular calcium concentration that would perturb cell metabolism. The energy-utilizing component of this model consists of the calcium transporters, which are located on the basolateral membrane, extruding calcium against a large electrochemical gradient. On the basis of this model, investigations of calcium transport in crustacean tissues have attempted to identify the presence of a calcium transporter whose activity correlates with periods of calcium fluxes.

Ca^{2+}-ATPase

While the Ca^{2+}-ATPase is considered to be the transport mechanism in many other tissues, attempts to correlate its activity with periods of Ca^{2+} flux in crustacean tissues have been unsuccessful. In *Callinectes sapidus* acclimated to sea water, Ca^{2+}-ATPase activity in the gills was equal at all stages of the moult cycle (Cameron, 1989). Epithelial Ca^{2+}-stimulated ATPase activity increased after the moult, although the increase was not proportional to the increase in fluxes (Cameron, 1989). Morris and Greenaway (1992) found a Ca^{2+}-stimulated ATPase with high affinity (K_m 6–35 $\mu mol\,l^{-1}$) in *Leptograpsus variegatus* that did not increase at postmoult and was therefore considered to be primarily for intracellular regulation. A Ca^{2+}-stimulated ATPase with a similar K_m (4–9 $\mu mol\,l^{-1}$) is found in the gills of the land crab *Birgus latro*, where it probably functions as a calcium transporter during urine reprocessing (Morris *et al.* 1991). Unfortunately, the method of measuring Ca^{2+}-ATPase is beset by difficulties, making it difficult to distinguish calcium-translocating activity from the activity of other ATPases that are stimulated by calcium (Walters, 1990). Alkaline phosphatase activity is particularly dependent on calcium and it is possible that much of the Ca^{2+}-stimulated ATPase activity with a high K_m and pH maximum, such as that found in sea water-acclimated *Callinectes sapidus*, may represent alkaline phophatase.

Na^+/Ca^{2+} exchange

A second transporter that could be involved in transepithelial calcium movements is the Na^+/Ca^{2+} exchanger that operates in transepithelial movement of calcium in tissues of some organisms (e.g. Flik *et al.* 1990). Roer (1980) used various transport inhibitors to demonstrate the dependence of calcium transport on substances that affect sodium transport, suggesting a role for Na^+/Ca^{2+} exchange in addition to Ca^{2+}-ATPase in the movement of calcium from the blood to the carapace. Similar conclusions were reached by Towle and Mangum (1985), who found an increase in Na^+/K^+-ATPase activity in the carapace epithelium of blue crabs after the moult. Na^+/Ca^{2+} exchange has yet to be conclusively demonstrated in crustacean tissues, however, and its role in transepithelial transport of calcium in crustaceans remains speculative. Measurements of the electrochemical gradient across the carapace epithelium have not been made and it is not clear whether movements are by passive or active mechanisms. Precipitation of calcium into the more alkaline carapace compartment (Cameron and Wood, 1985) may lower the calcium activity in this compartment and create an electrochemical gradient favouring passive movement from the blood to the carapace.

Ca^{2+}/H^+ exchange

Ahearn and Franco (1990) recently demonstrated that calcium can be transported by the Na^+/H^+ antiporter in crustacean antennal glands, suggesting a role for this transporter in the overall calcium regulation of crustaceans. This transporter is also located in the gills of crustaceans (Shetlar and Towle, 1989), where calcium uptake correlates well with apparent hydrogen excretion.

Conclusions

The gill and carapace epithelia of crustaceans obviously have fluxes of calcium that vary considerably in magnitude and direction according to moult stage. The similarity of calcium concentrations in the blood and the high rates of calcium uptake after the moult in various species suggest that the reliance on calcium is equally high for crustacean species that live in environments with a broad range of calcium availabilities. The mechanisms responsible for calcium regulation probably vary according to species and habitat, but data suggest that changes in the electrochemical gradient, permeability and active transport activity are all important. The electrochemical gradient depends on the particular conditions and can therefore change rates of passive fluxes. Permeability to calcium appears to vary with habitat, as it does for other ions. Finally, changes in active transport must also be responsible for some of the changes in flux rates, particularly after the moult. Despite the importance of calcium in the life cycle of crustaceans, there is a notable lack of information on the cellular mechanism of active transport and it is clear that studies of calcium regulation in crustaceans should concentrate on this aspect.

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