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CALCIUM BALANCE AT THE PREMOULT STAGE OF THE FRESHWATER CRAYFISH *AUSTROPOTAMOBIUS PALLIPES* (LEREBOULLET)

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SUMMARY

The premoult stage in *Austropotamobius pallipes* is characterized by a net loss of calcium which increases from D_0 to a maximum of 0·83 μmoles/g/h at D_4. The concentration of ionized calcium in the haemolymph does not increase during the premoult stage although there is an increase in complexed calcium. The electrochemical gradient across the body surface is similar to that at the intermoult stage and favours calcium outflux. Possible routes for calcium net loss have been discussed and a mechanism for elimination of calcium has been proposed.

INTRODUCTION

The changes in calcium metabolism involved in preparation for the moult in aquatic reptantian crustacea have been summarized by Passano (1960), Carlisle & Knowles (1959) and Travis (1960*a*). *Austropotamobius pallipes* appears to conform to the general pattern described by Passano. During the premoult stage calcium is removed from the old exoskeleton but only a small amount of this is retained after the moult, largely in the gastroliths (Chaisemartin, 1962, 1964, 1967). Gastrolith structure and formation in another crayfish, *Orconectes virilis*, has already been described (Travis, 1960*b*) and is not investigated in this paper. Most of the calcium removed during the premoult period in *Austropotamobius* is lost to the medium in soluble form (Chaisemartin, 1967) as is the case in marine decapods (Robertson, 1937; Travis, 1955). In the latter part of the premoult period (D_{3-4}) the concentration of calcium in the haemolymph of *Austropotamobius* rises (Chaisemartin, 1967; Greenaway, 1974) but it is important to remember that the concentration of ionized calcium and not total calcium concentration is the significant physiological parameter and this has not yet been measured. In the marine decapod *Panulirus argus*, the concentration of calcium in the urine rises in the premoult stage, reaching a level 20% above normal (Travis, 1955), but no data are as yet available for freshwater decapods. Chaisemartin (1967) found the potential difference across the epithelium of isolated podobranchs of *Austropotamobius* was lower at the premoult than at the intermoult stage, thus favouring calcium loss to the medium. Potential-difference measurements performed on isolated gill preparations, however, cannot safely be applied to intact animals (Greenaway, 1972) and measurements of the potential difference across the

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body surface of intact animals have, therefore, been made in this investigation determine whether such a shift in potential occurs in the intact crayfish.

The object of this work was to provide a detailed quantitative picture of the changes in calcium balance in *Austropotamobius* during the premoult period. Particular attention has been paid to points on which knowledge is poor, i.e. the activity of calcium in the blood and the route and mechanism of calcium loss from the body.

MATERIALS AND METHODS

Animals were collected and maintained as described previously (Greenaway, 1972). Crayfish kept in the laboratory usually began moulting in March. In the field several distinct moulting periods were observed; in June (males and non-ovigerous females), in late July/early August (females which did not moult in June) and in late August/early September (males and females). Moulting was synchronized, all participants moulting within a two-week period.

Experiments were carried out at 10 ± 1°C, but between measurements experimental animals were kept at 15°C in order to avoid lengthening the premoult by low temperatures. Animals preparing to moult were identified by calcium-balance experiments (Greenaway, 1974). In addition early premoult stages D_0 and D_1 could be identified, in small animals, by examining the setae on the uropods and telson (Stevenson, Guckert & Cohen, 1968) and crayfish at the later stages D_{3-4} were recognizable by flexibility of the branchiostegites and the appearance of the ecdysial line along the branchiostegite margin.

Calcium concentrations were measured using an E.E.L. 240 atomic absorption spectrophotometer. Ionic calcium was measured with an Orion calcium electrode.

RESULTS

Calcium balance

At the late intermoult stage, crayfish show a persistent low rate of net calcium loss to the medium of about 0·046 μmoles/g/h (Greenaway, 1972). This rate of net loss was increased from D_0 onwards and reached a very high level in animals at later premoult stages (D_{3-4}) (Fig. 1). The maximum net loss rate was observed a few days before the moult, and frequently a small reduction in loss rate occurred in the remaining days before ecdysis. In Table 1 some values for maximal net calcium loss are presented. The mean value of 0·83 μmoles/g/h is about 36 times the calcium efflux in early C_4 animals and 20 times the net calcium loss rate in late C_4 crayfish (Greenaway, 1972). The maximum net loss value of 1·495 μmoles/g/h is 65 times the intermoult efflux. Chaisemartin (1967) has also measured net loss in premoult (D_{3-4}) crayfish and gives a value of 0·89 μmoles/g/h, which is in close agreement with the mean value given in Table 1.

Sodium balance

Measurements of the sodium loss rate to sodium-free artificial tap water have been made for premoult crayfish at stage D_4 (Table 2). The normal loss rate for animals at the intermoult stage is approximately 0·15 μmoles/g/h (Shaw, 1959), so clearly permeability to sodium is doubled in the late premoult stage. This increased sodiu

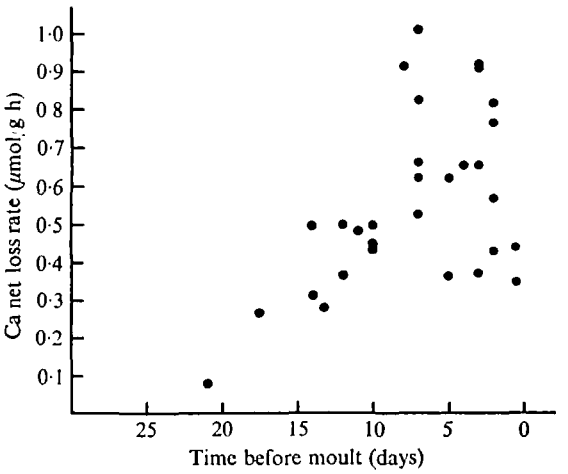

Fig. 1. The rate of net loss of calcium from crayfish during the premoult period. Values for several animals are included.

Table 1. *Maximum values for calcium net loss (μmoles/g/h) from crayfish at the late premoult stage*

0·653 0·914 1·470 1·016 0·915 0·661 0·621 0·703 0·904 0·618 0·659 0·823

Mean 0·830 ± 0·068 S.E.

Table 2. *Sodium loss rates from late premoult stage crayfish*

Stage	Time before moult (days)	Na loss rate (μ moles/g/h)
D_4	2	0·329
D_4	2	0·274
D_4	2	0·389
D_4	2	0·422
D_4	3	0·351
D_4	3	0·330
D_4	3	0·191
D_4	3	0·328
D_4	3	0·297
Mean		0·323 ± 0·02 S.E.

turnover may perhaps be indicative of a slight increase in permeability of the exoskeleton at this stage, although the increase in calcium net loss was ten times greater than that of sodium.

Total calcium loss in premoult

From Table 3 it is apparent that 83% of the total calcium content was lost during stages D (34%) and E (66%). The newly moulted crayfish contained only 17% of its calcium content at the previous intermoult stage. Of this calcium 60% is in the gastroliths and has been shown to originate in the old exoskeleton (Chaisemartin, 1967). Thus, much of the calcium removed from the old exoskeleton was passed into the body and 34% was stored. Values given in Table 3 agree well with the data of Chaisemartin (1967) and the total calcium loss during stages D and E is also similar that in marine decapods (Passano, 1960).