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THE HAEMOLYMPH AS A STORAGE SITE FOR CUTICULAR IONS DURING PREMOULT IN THE FRESHWATER/LAND CRAB *HOLTHUISANA TRANSVERSA*

BY STEPHEN SPARKES AND PETER GREENAWAY*
School of Zoology, University of New South Wales, P.O. Box 1, Kensington, N.S.W., Australia 2033

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SUMMARY

Holthuisana transversa (von Martens) utilizes the haemolymph as a storage site for ions reabsorbed from the cuticle in premoult. 66 % of the total body calcium, magnesium and phosphate from the preceding intermoult stage was stored in the haemolymph in premoult. Almost all of the calcium and phosphate and 73 % of the magnesium in the haemolymph was located in microspherules of average diameter 0·25 μm. The concentration of total calcium in the haemolymph in premoult was 150 times the normal intermoult value (13·2 mmol l^{-1}) but the activity of calcium in the haemolymph decreased from an intermoult value of 4·52 mmol l^{-1} to 3·37 mmol l^{-1} in premoult. Postmoult crabs absorbed calcium from the water at a high rate and showed a high affinity for calcium ions; K_m = 0·105 ± 0·013 mmol l^{-1} Ca.

INTRODUCTION

Marine decapods lose a large proportion of their total body calcium when they moult (Passano, 1960). Most of the calcium retained (10–20 % of the total at the preceding intermoult stage) is derived from the demineralization of the old exoskeleton and stored in some part of the body. The calcium is rapidly regained by absorption of calcium ions from sea water (Robertson, 1937, 1960). The stored calcium may be a side-product of the animal's attempt to conserve phosphate ions which are in short supply in sea water (Sather, 1967). In freshwater decapods, storage of resorbed ions is of a similar order to that in marine species and the main adaptation to fresh water is an increase in the affinity of the calcium uptake mechanism (Greenaway, 1972, 1974*a*,*b*,*c*, 1983). The terrestrial mode of life necessitates increased storage of calcium as the animals no longer have access to the virtually unlimited supply of calcium available in water. Instead, they must obtain calcium from their diet and, to enable foraging activity after the moult, enough calcium must be retained to harden the new exoskeleton adequately. Thus in terrestrial amphipods and isopods,

* Send reprint requests to this author.
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storage is high (Graf, 1974, 1978) but no information is available for terrestrial decapods.

Several different storage sites for calcium have been identified in aquatic decapods.

(a) Midgut gland – e.g. marine crabs where calcium is stored as intracellular spherules of calcium phosphate (Robertson, 1937; Posner, 1977; Travis, 1955*a*).
(b) Gastroliths – discs of calcified tissue in the stomach walls of crayfish, lobsters and some crabs (Numanoi, 1939; Travis, 1963).
(c) Haemolymph – certain crabs e.g. *Sesarma dehaani* (Grapsidae) (Numanoi, 1939) and freshwater crabs of the family Sundathelphusidae.

Storage in cells of the midgut gland and in gastroliths has been studied intensively and the structure, formation and role of these stores is well understood (Travis, 1960, 1963; Robertson, 1937). However, the role of the haemolymph as a storage site for calcium has not been considered since the work of Numanoi (1939). He found that the concentration of calcium in the haemolymph of *Sesarma dehaani* rose from normal levels of 18·8 mmol l^{-1} at intermoult to 962·5 mmol l^{-1} in late premoult. The haemolymph at this point became opaque and milky in appearance. A similar phenomenon occurs in *Holthuisana transversa*, a freshwater/land crab found in semi-arid regions of Australia. Here surface water dries rapidly and for much of the year the crab lives in a dry burrow and is relatively inactive (MacMillen & Greenaway, 1978). Moulting occurs in water during the aquatic phase. The object of this work was to assess the role of the haemolymph as a storage site for cuticular ions during premoult.

MATERIALS AND METHODS

The habits, distribution and systematics of *Holthuisana* (*Austrothelpusa*) *transversa* (von Martens) were described by Bishop (1963) and Bott (1970).

H. transversa were collected from Rossmore Station, Bourke; Avoca Station, Gulargambone; or Glenelvyn Station, Lightning Ridge, N.S.W. *Holthuisana* (*Austrothelphusa*) *agassizi* (Rathbun) and *Holthuisana* (*Austrothelphusa*) *valentula* (Riek) were collected from the Cooktown and Coen areas of N. Queensland respectively. The crabs were kept in glass jars containing a few centimetres of an artificial medium (ATW) similar to Sydney tap water (Greenaway, 1980) or pond water and fed on fish-based cat biscuits.

Premoult ion storage

Total body calcium and magnesium were determined after ashing intermoult crabs in platinum crucibles at 450 °C. The ash was then dissolved in concentrated nitric acid and the excess acid boiled off. The concentrations of calcium and magnesium were measured with a Varian AA5 atomic absorption spectrophotometer and samples and standards contained 7·2 mmol l^{-1} $LaCl_3$.

Ion contents of soft crabs (1–2 days postmoult) and their cast exoskeletons were determined similarly. The ion contents of these animals just before their moult were estimated using the data for calcium and magnesium contents of intermoult crabs (Fig. 1) and their premoult wet weights. These were then compared with the sum of the values for postmoult crabs and their exuviae to determine the amount of calcium and magnesium retained through the moult.

Total concentrations of calcium, magnesium and inorganic phosphate in the haemolymph were measured during intermoult, late premoult and early postmoult stages. Haemolymph was obtained using the method of Greenaway & MacMillen (1978). Haemolymph at late premoult contained calcareous microspherules and these were dissolved with concentrated HCl prior to analysis for total ions. Concentrations of inorganic phosphate and total phosphates (premoult crabs only) were measured using the methods of Baginski, Foa & Zak (1967).

Ionized calcium in the haemolymph was measured using an Orion model 93-20 calcium electrode, model 401 specific ion meter and microsample dishes. The electrode was calibrated with $CaCl_2$ standards containing sodium, potassium and magnesium (as chlorides) in the concentrations at which they occurred in the haemolymph.

The activity of calcium was calculated using the activity coefficient from the Davies equation for solutions $>0{\cdot}2\,\text{mol}\,l^{-1}$ ionic strength (Zanker, 1972):

$$\frac{-\log_{10} fi}{Z^2} = \frac{0{\cdot}511 \times \sqrt{\mu}}{+1{\cdot}5\sqrt{\mu}} - 0{\cdot}2\mu,$$

where fi = activity coefficient, Z = the valence and μ = ionic strength determined from the plasma concentrations.

The pH of the haemolymph was measured anaerobically using a Radiometer G297/G2 capillary electrode and Radiometer blood-gas analyser.

Calcareous microspherules were separated from the fluid fraction of the haemolymph of premoult crabs by centrifugation, at 12 000 ***g***, under mineral oil. The concentration of calcium not in microspherules (plasma calcium) was measured in samples of the supernatant. The pellet was dried at 70 °C, ground and dissolved in 1 ml $0{\cdot}6\,\text{mol}\,l^{-1}$ HCl. The amount of carbonate in the pellet was then determined by titration with $0{\cdot}06\,\text{mol}\,l^{-1}$ NaOH to an endpoint of pH 7 using a Radiometer ETS822 Autotitrator. The calcium content of the titrated samples was measured and the ratio of calcium to carbonate determined.

The organic content of the microspherules was determined as follows. Microspherules were washed several times in $10\,\text{mmol}\,l^{-1}$ calcium activity standard, dried at 70 °C, weighed and ashed at 450 °C. Ash weight was then measured. The weight loss during ashing was taken to be organic material.

The volume of microspherules as a percentage of the volume of the haemolymph was measured by centrifugation in haematocrit tubes.

It was possible that calcium, magnesium or phosphate could be reabsorbed preferentially from the exoskeleton during premoult. To investigate this, analyses were carried out on skeletal material from intermoult crabs and fresh exuviae. Exoskeleton from the chelae and the carapace was cleaned and dried, ground to a powder and digested using the sulphuric acid-peroxide method of Allen, Crimshaw, Parkinson & Quarmby (1974). Levels of ions were determined as above.

Microspherules were prepared for study with a scanning electron microscope (JEOL JSM-35C). After separation from the haemolymph with $0{\cdot}2\,\mu$m Millipore filters, they were washed with $10\,\text{mmol}\,l^{-1}$ calcium activity standard, dried and coated with gold-palladium.

Net uptake of calcium by postmoult crabs

Individual crabs were placed successively in six different solutions of artificial tap water containing calcium at concentrations from 0·05–3·0 mmol l^{-1}. Samples were removed at 10 or 15 min intervals over a period of 1 h and analysed for calcium. The rate of net uptake of calcium was determined from the linear decrease in the concentration of calcium over each experimental period. The external concentration at which half-maximal calcium transport occurred (K_m) was then calculated by regression analysis from Lineweaver-Burk plots of the rate of net uptake of calcium at the various external concentrations of calcium (Sutcliffe, 1975).

RESULTS

Total body calcium and magnesium

Total body contents of calcium and magnesium in *H. transversa* at the intermoult stage were linearly related to body weight (Fig. 1). The slopes of the regression lines differed significantly between the sexes for both calcium ($P < 0{\cdot}001$, *t* test) and magnesium ($P < 0{\cdot}02$, *t* test). The contents of calcium and magnesium were higher in male crabs than in female crabs of equivalent weight. For example, male crabs of 30 g weight contained 24 % more calcium and 17·4 % more magnesium than did females. The mean ratio for total body content of Ca and Mg (26·3) was largely independent of body size and sex. In *Austropotamobius pallipes* a much higher Ca: Mg ratio (53) was reported (Greenaway, 1974*c*), indicating a total body content of Mg about half that found in *H. transversa*.

Calcium budget during the moult cycle

The total body contents of calcium, magnesium and phosphate were measured in

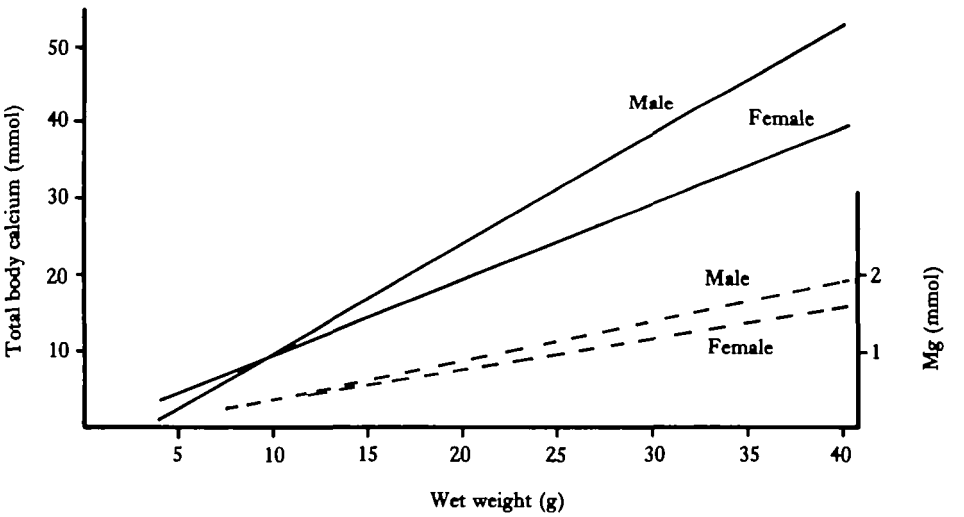


Fig. 1. Calcium content (——) and magnesium content (– – –) of intermoult crabs. Regression equations were: Ca males y(mmol) = 1·44x−4·474; Ca females y = 0·995x−0·324; Mg males y = 0·052x−0·151; Mg females y = 0·0412x−0·039.

Table 1. *Mean total ion content of 10 postmoult crabs and their exuviae*

	Premoult weight (g)	Soft crab (mmol)	Exuviae (mmol)	Total content (mmol)	% in soft crab ± S.D.	Estimated intermoult content (mmol)	Ca: ion ratio
Ca	16·1	11·11	5·93	17·04	65·6 ± 4·2	18·71	—
Mg	16·1	0·375	0·215	0·59	64·4 ± 5·1	0·71	29·4
PO_4	16·1	1·09	0·52	1·61	68·5 ± 5·5	—	10·9
					$\bar{X} = 66·2$		

soft crabs and their exuviae and compared with values for ion contents of intermoult crabs estimated from Fig. 1 and Table 1.

The soft crab contained approx. 66 %, and the exoskeleton 34 %, of each ion and there were no significant differences ($P > 0·1$, t test) in the relative abundance of these ions between soft crabs and their exuviae. In the one specimen of *H. agassizi* examined 74·9 % of the total postmoult calcium and magnesium was in the soft crab and 25·1 % in the exuviae.

The mean calcium content at intermoult (estimated from Fig. 1) was slightly higher [1·4 % (range −22·8 % to +22·1 %)] than the calcium content of the soft crab plus its exuviae. Thus little if any calcium appeared to be lost during premoult. However, the mean content of Mg at intermoult was 26·3 % greater (range 10·2 % to 42·8 %) than for soft crabs plus their exuviae, which indicated a loss of Mg in solution from the crabs during premoult. This was reflected in the mean Ca: Mg ratio in postmoult crabs which was significantly higher than found in intermoult animals. The calcium: phosphate ratio in premoult crabs was 10·9.

Haemolymph ion concentration

The mean concentration of total calcium in the haemolymph of intermoult crabs was 13·2 ± 1·3 S.D. mmol l^{-1} ($N = 32$), which is similar to reported values for other crustaceans e.g. *Carcinus maenas* (12·8 mmol l^{-1}, Greenaway, 1976) and *Austropotamobius pallipes* (11·7 mmol l^{-1}, Greenaway, 1974*c*). Ionized calcium (10·6 ± 1·4 S.D. mmol l^{-1}, $N = 13$) comprised 79·2 % of the total calcium, indicating

Table 2. *Haemolymph calcium concentrations (mmol l^{-1}) in premoult and postmoult* Holthuisana

	Total Ca concentration				PREMOULT			
	65–40 h premoult	40–18 h premoult	18–0 h premoult	0–12 h postmoult	Plasma total	Plasma ionized	Plasma calcium % ionized	% of calcium in microspherules
Mean	471·4	1010	1640	126	23·8	7·6 (5·5–11·0)*	29·3	97·8
S.D.	114	174	279	147	2·5	1·8	5·7	1·2
N	8	9	12	8	10	8	6	10

* Range

Table 3. *Haemolymph phosphate concentrations (mmol l^{-1}) through the moult cycle of* Holthuisana transversa*

	Intermoult concentration	Premoult concentrations			0–12 h postmoult	Ca: PO_4 in premoult	
		65–40 h	40–18 h	18–0 h		inorganic PO_4	total PO_4
Mean	0·035	31·8	76·0	102·6	2·5	14·6	14·5
± S.D.	0·018	4·1	10·0	25·0	2·6	3·2	1·9
N	4	7	7	7	6	23	10

* Figures are for inorganic phosphate (except last column).

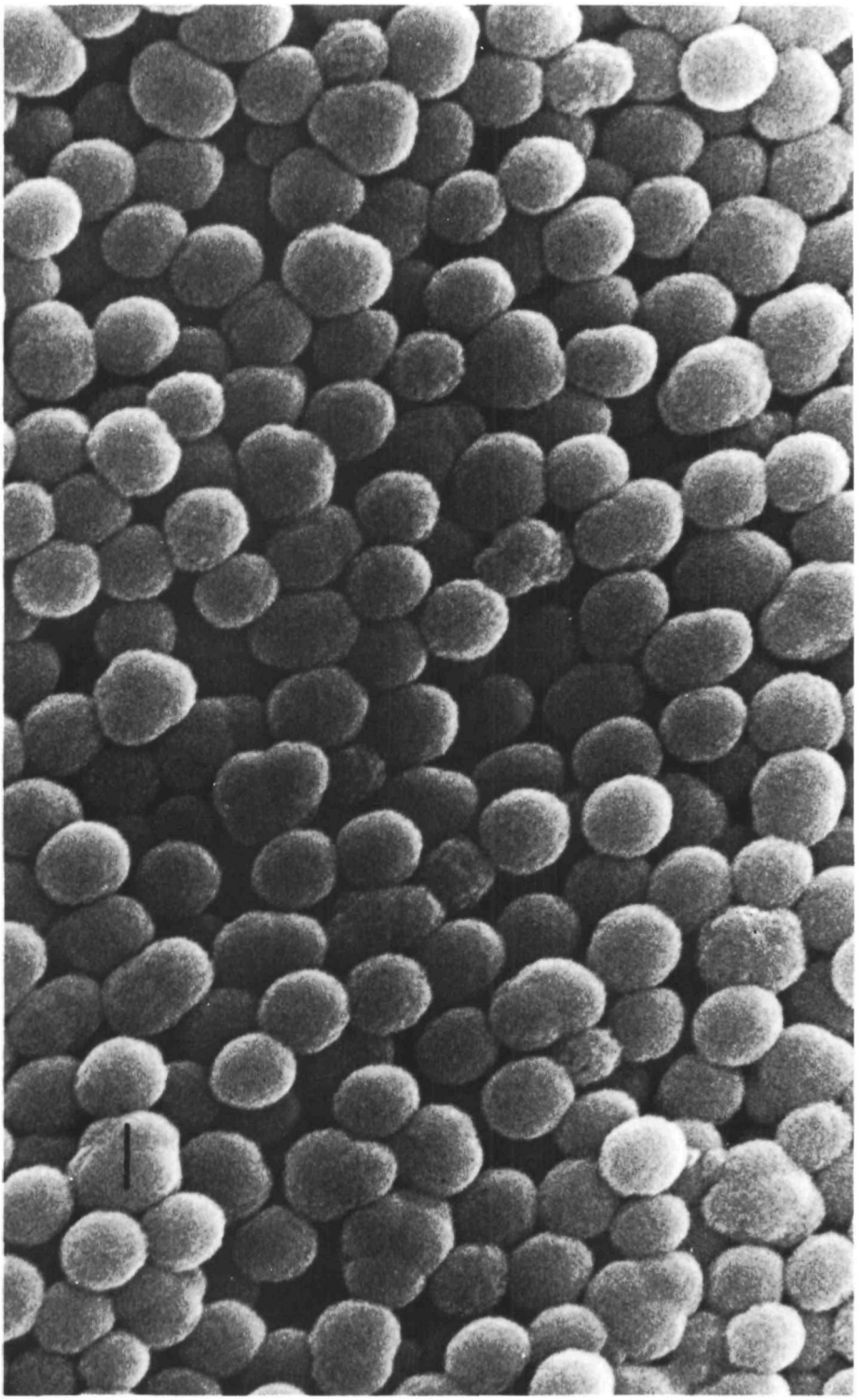
that about 20 % of the haemolymph calcium was in non-ionic form. This non-ionized calcium was probably bound to blood proteins or complexed with inorganic, and small, organic anions (Greenaway, 1971).

Approximately 3 days before the moult the total calcium content of the haemolymph increased dramatically (Table 2). The haemolymph first became cloudy and then, as the calcium concentration continued to rise, turned creamy white. Although the mean calcium concentration of the haemolymph in the 18 h before moult was 1·64 mol l^{-1}, a staggering 124 times the intermoult level, this underestimated the maximum concentration of calcium attained. The highest value measured in the haemolymph of *H. transversa* in premoult was 2·024 mol l^{-1} (153 times the intermoult mean) and it is likely that the haemolymph calcium concentration of all individuals approached 2 mol l^{-1} just prior to moult. The same dramatic increase in haemolymph calcium concentration in premoult also occurred in *H. agassizi* and *H. valentula*, where the maximum recorded calcium concentrations were 2·624 mol l^{-1} and 2·224 mol l^{-1} respectively. After the moult the total haemolymph calcium dropped rapidly and a mean concentration of 125 mmol l^{-1} was measured 12 h after ecdysis.

On centrifugation, samples of haemolymph from crabs at premoult separated into a fraction containing microspherules and a clear plasma. The mean value for plasma calcium during the entire 'white blood' period was 23·8 mmol l^{-1} (Table 2). During this period plasma calcium accounted for only 2·2 % of the total calcium in the haemolymph. At the same time ionized calcium dropped from its intermoult value of 10·6 mmol l^{-1} to 7·6 mmol l^{-1}; thus only 31·9 % of plasma calcium was ionized in premoult. This decrease may have been due to an increase in the concentration of binding proteins and complexing ions in the haemolymph at that time or to the abstraction of calcium for production of microspherules. The activity of calcium in the haemolymph of intermoult crabs was calculated to be 4·52 mmol l^{-1} and in late premoult fell to 3·37 mmol l^{-1}.

The concentration of inorganic phosphate in the haemolymph was measured at intermoult, during the 3 days preceding ecdysis, and 12 h postmoult (Table 3). The

Fig. 2. Microspherules from the haemolymph of *Holthuisana transversa* in late premoult. Approximate magnification ×90 000. Bar is approximately 100 nm. Note several instances where microspherules appear to have fused.



mean intermoult value was 0·035 mmol l^{-1} but this level increased in the 3 days prior to moult and reached a mean value of 102·6 mmol l^{-1} (×2930 increase) in the 18 h preceding moult. The maximum recorded haemolymph concentration at this time was 134·9 mmol l^{-1}, 3850 times the intermoult mean. The inorganic phosphate concentration of the haemolymph dropped rapidly in postmoult. There was no significant difference between the ratios of calcium: inorganic phosphate or calcium: total phosphate in the 3 days before ecdysis indicating that the bulk of total phosphate was in the inorganic form (Table 3).

The mean concentration of magnesium in the haemolymph of intermoult *H. transversa* was 2·7 ± 0·53 S.D. mmol l^{-1} ($N = 10$). During the 18 h before moult the total concentration of magnesium in the haemolymph reached a mean value of 47·0 ± 12·3 S.D. mmol l^{-1} ($N = 8$) (17 times the intermoult mean) with a maximum recorded concentration of 68·1 mmol l^{-1}. Plasma magnesium accounted for 27·1 % ± 5·5 S.D. of the total haemolymph magnesium at this time. The calcium: magnesium ratio was 31·6 ± 4·9 S.D. ($N = 10$).

The mean pH of the haemolymph of crabs in late premoult was 8·031 ± 0·039 S.E.M. ($N = 7$).

Haemolymph microspherules

Microspherules separated from the plasma, as described above, and examined with a scanning electron microscope appeared more or less spherical in shape with a diameter of 0·25 μm. Some appeared to have been formed by coalescence of several smaller microspherules (Fig. 2).

The ratios of ionic concentrations in the microspherules are listed in Table 4. The calcium: carbonate and calcium: magnesium ratios were determined specifically for

Table 4. *Composition of microspherules*

	Ca: CO_3	Maximum possible % of Ca as $CaCO_3$	% Organic matter	Ca: PO_4	Ca: Mg
Mean	1·15	87·3	21·7	14·5	42·9
± S.D.	0·0	3·3	1·2	1·9	7·6
N	10	10	7	10	10

Table 5. *Concentrations of ions in intermoult exoskeleton (mmol g^{-1} dry weight) and the exuviae of* Holthuisana transversa

	Ca	Phosphate	Mg	Ca: PO_4	Ca: Mg*
Exoskeleton					
Mean	5·7	0·46	0·17	12·6	33·9
± S.D.	0·23	0·07	0·02		
N	11	11	10	11	10
Exuviae					
Mean	5·66	0·51	0·23	11·6	26·9
± S.D.	0·44	0·11	0·06		
N	12	12	11	12	11

* Intermoult exoskeleton and exuviae are significantly different ($P < 0·05$) using modified *t* test.

Table 6. K_m *(mmol* l^{-1} *Ca) and* K_{max} *(μmol* g^{-1} h^{-1}*) values for* Holthuisana transversa *during postmoult absorption of calcium*

Number	K_m	K_{max}	Weight (g)
1117	0·123	6·006	4·36
1099	0·054	3·453	5·93
1044	0·137	2·412	29·44
1049	0·136	5·236	11·54
1034	0·089	2·032	23·24
1022	0·091	2·816	13·72
$\bar{X}$ ± S.E.M.	0·105 ± 0·013	—	—

the microspherules, while the calcium: phosphate ratio included any phosphate in the plasma as well. Most calcium was present as carbonate with $CaCO_3$ comprising up to 87·3%. Organic matter accounted for 21·7% of the total dry weight of the microspherules.

The maximum volume of microspherules, as a percentage of total haemolymph volume, was 30·3. The volume of microspherules and concentration of total calcium in the haemolymph were linearly related ($y = 68{\cdot}3x - 30{\cdot}5$, $r^2 = 0{\cdot}99$, where x is the volume of microspherules as a percentage of haemolymph volume and y is the total concentration of calcium in the haemolymph in mmol l^{-1}).

Mobilization of exoskeletal ions during premoult

Considerable reabsorption of ions and inorganic material occurred from the old exoskeleton during premoult. This process was relatively indiscriminate but magnesium was significantly higher in the exuviae and clearly was not reabsorbed to the same extent as other ions (Table 5). The higher Ca: Mg ratio in the microspherules confirms this. Thus during premoult the exoskeleton became thinner but the relative proportions of ions and organic material did not change greatly.

Net uptake of calcium during postmoult

For some hours after the moult *H. transversa* showed a small net loss of calcium to the medium but within 24 h calcium balance became positive. If left in a limited volume of ATW the crabs reduced the calcium concentration to a low level, mean = 5·2 μmol l^{-1} (range < 1–8·5 μmol l^{-1}, $N = 10$). The affinity for calcium was quite high with $K_m = 0{\cdot}105 \pm 0{\cdot}013$ S.E.M. mmol l^{-1} (Table 6). There were insufficient data in Table 6 to establish any significant relationship between K_{max} and body size. However, more data were available for the rate of net uptake from 0·93 mmol l^{-1} Ca and here the weight-specific rate of net uptake decreased with increasing body size according to the relationship net uptake (μ mol g^{-1} h^{-1}) = 11·71 $m^{-10{\cdot}529}$.

DISCUSSION

Just prior to ecdysis, the concentration of total calcium in the haemolymph of *Holthuisana* increased dramatically to about 150 times the intermoult value. The

maximum concentration appeared to be species-specific although more data are required for *H. agassizi* and *H. valentula*. In other decapod crustaceans the premoult calcium concentration may reach twice the intermoult value. For example, in *Panulirus* there was an 86 % increase (Travis, 1955*b*) and in *Sesarma catenata* a 66·5 % increase (Hecht, 1975) but only in the two species of *Sesarma* studied by Numanoi (1939) was there an elevation of similar magnitude to that found in *Holthuisana*. Although the concentration of total calcium in the haemolymph increased in premoult *H. transversa*, the concentration of ionized calcium actually fell from an intermoult value of 10·6 mmol l^{-1} to 7·6 mmol l^{-1}. The concentrations of magnesium and phosphate in the haemolymph followed similar patterns to that for calcium; they increased greatly in premoult crabs.

The haemolymph acted as a premoult storage site for all major cuticular ions. Approximately 66 % of the total body calcium at the preceding intermoult stage was retained through the moult in *H. transversa* whilst in *H. agassizi* and *H. valentula* even greater storage was probable. This compares with 10–20 % retention in most decapod crustaceans; e.g. *Austropotamobius* lost 83 % of its total calcium content during the moult (Greenaway, 1974*a*). It is possible to calculate how much of the calcium conserved through the moult was stored in the haemolymph. A 10 g male crab with a blood volume of 3·2 ml (Greenaway, 1980) and a concentration of total calcium in the haemolymph of 2 mol l^{-1} would have 6·4 mmol in the haemolymph in premoult. At the intermoult stage such a crab would be expected to contain 10·18 mmol of calcium of which 66 % (6·7 mmol) (Fig. 1, Table 1) would be conserved through the moult. Thus, most of the calcium retained through the moult (95·2 %) was stored in the haemolymph. The remaining 4·8 % probably represented general body calcium although possibly some was stored in the midgut gland as reported for the amphipod *Gammarus pulex* (Wright, 1980) and certain crabs (Robertson, 1937; Becker, Chen, Greenawalt & Lehninger, 1974). Alternatively, it might simply represent an artifact of the calculation based on mean data.

Storage of calcium in the haemolymph of crabs has only been reported previously for two species of grapsids, *Sesarma dehaani* and *S. hematocheir* (Numanoi, 1939) and there is no indication that this pattern is typical of the family. For example, Hecht (1975) found only a small rise in total calcium in the haemolymph of *S. catenata* during premoult. All three species of *Holthuisana* which were examined used the haemolymph as a storage site but information is lacking for other families of freshwater crabs. No similar phenomenon has been reported in other groups of Crustacea.

The reabsorbed cuticular ions were carried in the haemolymph as discrete microspherules. These microspherules did not appear crystalline in nature and were probably composed of amorphous calcium carbonate and phosphate with a small amount of magnesium. They were similar in shape to the microspherules produced in the hindgut caeca of the amphipod *Orchestia cavimana* but with a diameter only a quarter of the size (Graf, 1971) The small size of the microspherules in *H. transversa* is presumably necessary to allow their passage through the narrowest capillaries (2–10 μm, Taylor & Greenaway, 1979) without impeding flow of haemolymph.

The number of spheres in a late premoult crab was estimated from their diameter (0·25 μm) and the volume of the microspherules as a fraction of the haemolymph

volume (30·3 %). The value obtained was $37{\cdot}04\times10^{12}$ microspherules ml^{-1} haemolymph. The rate of production of microspherules was calculated to be $45{\cdot}7\times10^{6}\,g^{-1}$ wet weight s^{-1}, on the assumption that the rate was constant during the 3 days before ecdysis. As haemolymph volume was not measured in premoult crabs the effect of such large numbers of microspherules on the fluid volume is not known. The microspherules would certainly increase the density and viscosity of the haemolymph considerably and thereby increase the energy required to circulate the haemolymph. The site of formation of the microspherules is unknown. It seems unlikely that they are formed in the haemolymph but a preliminary investigation of the hypodermis and midgut gland of premoult crabs as possible sites of manufacture (using transmission electron microscopy) was negative and a more thorough study is required. Another potential production site is the hindgut caecum which is concerned with synthesis of calcium granules in *Orchestia* (Graf & Michaut, 1977), but this is a relatively small organ and probably has insufficient cells to achieve the necessary rate of production of microspherules.

Since the microspherules are suspended in the haemolymph it would be expected that the haemolymph should be saturated with respect to calcium carbonate. At intermoult, the mean haemolymph pH was 7·34 (Greenaway, Bonaventura & Taylor, 1983) and the carbonate level and the ionic product were very low. The intermoult ionic product was a factor of ten lower than the apparent solubility product for calcite in sea water of 12‰ (salinity equivalent of the osmotic pressure of the haemolymph of *Holthuisana*; MacIntyre, 1965) which implied that the blood was not saturated with respect to calcium carbonate at this stage. In premoult crabs, the pH rose to 8·031 which increased the concentration of carbonate so that the ionic product exceeded the apparent solubility product, indicating saturation of the haemolymph with respect to calcite. It is difficult to predict how close this calculated example is to the real situation since the microspherules are probably composed of amorphous $CaCO_3$ and hence have a different solubility product. Also organic ions, such as citrate, in the haemolymph may alter this product.

After ecdysis the concentration of stored ions in the haemolymph decreased rapidly as they were remobilized to calcify the new exoskeleton. Their concentration in the haemolymph approached the intermoult values within 12 h, indicating an extremely rapid rate of calcification. Calcium incorporation was calculated to be in excess of $50\,\mu mol\,g^{-1}\,h^{-1}$. Marine and freshwater decapods relying on absorption of calcium from the water have rates of calcium deposition almost an order of magnitude lower (Greenaway, 1974*c*, 1983).

Although *Holthuisana transversa* retains a much larger proportion of its body calcium through the moult than do other aquatic decapods it still faces a considerable shortfall in the postmoult requirement for calcium, probably equivalent to 50 % of its premoult calcium content. This could be made good from the water and/or the food, especially if the exuviae were eaten. In the field *Holthuisana* has not been observed to eat its exuviae, although this is common under the holding conditions in the laboratory. The high rate of net uptake of calcium from the water by postmoult crabs strongly suggests that this source is of major importance in the supply of calcium for the completion of recalcification. The rate of net uptake from ATW (0·9 mmol l^{-1} Ca) was similar to that found for crayfish of the same size (although measurements in th

latter were made at 10 °C; Greenaway, 1974*c*) and to rates from sea water (10 mmol l^{-1} Ca) for *Callinectes* and *Carcinus* (Greenaway, 1983). The affinity of the calcium transport mechanism in *Holthuisana* (K_m 0·105 mmol l^{-1}) was slightly higher than found in *Austropotamobius* (0·13 mmol l^{-1}) (Greenaway, 1974*c*) and considerably greater than in *Gammarus pulex* (0·3 mmol l^{-1}) (Wright, 1979), the only other freshwater crustaceans for which data are available.

In summary, crabs of the genus *Holthuisana* store a large proportion of their intermoult cuticular ions in the haemolymph as microspherules during premoult. This allows extremely rapid hardening of the new exoskeleton and therefore reduces their dependence on the availability of free water. In the field in summer, free water may evaporate before recalcification is complete or the volume of water available may be very small and storage of such a large proportion of body calcium through the moulting period would ensure that recalcification proceeded at a maximal rate independent of the calcium concentration of the water. Additionally, calcium reserves are large enough to ensure survival of the crab even if complete calcification were prevented by premature drying of water.

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