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THE EFFECT OF CALCIUM ON CADMIUM UPTAKE BY THE SHORE CRAB *CARCINUS MAENAS*

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SUMMARY

The accumulation of cadmium by the shore crab *Carcinus maenas* (L.) is to some extent dependent upon the calcium concentration of the external medium. This effect is apparently independent of the overall salinity of the external medium and may at least partially explain previous reports of a 'salinity effect'.

Haemolymph cadmium has a highly significant inverse relationship with the external calcium concentration. This effect is less obvious with other tissues, although the whole body cadmium has a significant inverse correlation with the external calcium concentration. Both the haemolymph and gill show a significant inverse relationship between tissue cadmium and calcium.

When postmoult animals were exposed to 20 μ-mol l^{-1} Cd in 100% s.w., high concentrations of both calcium and cadmium appeared in the haemolymph. Postmoult animals in cadmium-free sea water generally had a lower haemolymph calcium concentration than intermoult animals, and the rise in haemolymph calcium seen in the presence of cadmium may indicate some degree of competition for 'deposition sites' between these two metals.

INTRODUCTION

In earlier papers (Wright, 1977*a*, *b*) it was shown that cadmium uptake from a medium containing 20 μ-mol l^{-1} cadmium was faster in 50% sea water than 100% sea water. Uptake rates by whole animals were 0·51 μ-mol Cd (g wet wt)$^{-1}$ h^{-1} and 0·34 μ-mol Cd g^{-1} h^{-1} respectively. Results were similar to those reported for other euryhaline crabs exposed to dilute sea water (O'Hara, 1973*a*, *b*; Hutcheson, 1974).

The tissue mainly affected by salinity in this respect was the exoskeleton (as measured by analysis of the carapace) which also had the highest overall cadmium concentration (Wright, 1977*a*). A high cadmium concentration was also often found in the hepatopancreas. It therefore seemed likely that a relationship might exist between cadmium accumulation and the calcium status of the animal. It is known that the hepatopancreas stores calcium (and magnesium) probably as a reserve for the exoskeleton (Robertson, 1937, 1960).

Both calcium and cadmium exist as divalent ions in the free form although both have a tendency to form complexes, particularly with organic molecules such as proteins. Wright (1977*b*) found that almost all haemolymph cadmium eventually

became bound to haemolymph protein in *Carcinus*. The bound calcium fraction was generally between 20–30% of the total haemolymph calcium (Robertson, 1937; Greenaway, 1976; Wright, 1977*b*). Greenaway (1976) has shown that *Carcinus* is highly permeable to calcium, and it seems likely that a similar situation exists for cadmium.

In this paper the effect of external calcium concentration upon cadmium uptake is investigated at two different salinities. To gain greater insight into the 'calcium effect', some observations have also been made on cadmium accumulation by postmoult specimens.

MATERIALS AND METHODS

All animals were collected from Boulmer, Newton and Cresswell in Northumberland. Postmoult animals were collected during August 1975 and June 1976 and were experimentally matched with control animals of similar size and sex. As all these animals were collected from the field, it was not possible to be specific regarding the period since moulting. Description of moulting status was based therefore on the hardness of the shell. That of the softest animal had the consistency of thin paper and the animal was clearly within a few hours of moulting. There were two exceptions to this general classification and these are further described in the text.

Methods of analysis were mainly as described previously, although some modifications were employed. Acid digestion of tissue was done using a 30:1 double distilled nitric/perchloric acid mixture under conditions of strong light. Rate of oxidation was found to be significantly increased under such conditions (P. Lobel, personal communication). For some tissue calcium analyses, lanthanum chloride was used to offset anionic interference at a concentration of > 1 g l^{-1}.

For the first experiment an artificial sea water was used which contained 23·5 g NaCl, 0·66 g KCl, 3·9 g Na_2SO_4, 11 g $MgCl_2.6H_2O$, 0·02 g $SrCl_2$ and 0·15 g $NaHCO_3$ per l distilled water. The calcium concentration was varied by addition of $CaCl_2.2H_2O$ and this was subsequently checked by atomic absorption spectroscopy. This manipulation of the calcium concentration was set against a background of two different salinity regimes, 100% and 40% sea water. The external calcium concentration is hereafter referred to as a percentage of its normal seawater concentration (10 m-mol l^{-1}). Thus an experimental medium containing all the major ionic constituents in their normal seawater concentrations but having only 4 m-moles calcium per litre is designated 100% s.w., 40% Ca. The experimental media used were as follows: 100% s.w., 20% Ca; 100% s.w., 40% Ca; 100% s.w., 80% Ca; 100% s.w., 100% Ca; 40% s.w., 40% Ca; 40% s.w., 80% Ca; 40% s.w., 100% Ca and 40% s.w., 150% Ca. *All* experimental media contained 20 μ-mol Cd l^{-1}.

RESULTS

Batches of six animals were each exposed to one of the eight experimental media (see Methods). After 2 weeks the haemolymph, muscle, gill hepatopancreas and exoskeleton (carapace) were analysed for both cadmium and calcium.

Haemolymph cadmium concentrations are presented in Fig. 1 as a function of the external calcium concentration and reveal a highly significant inverse correlation

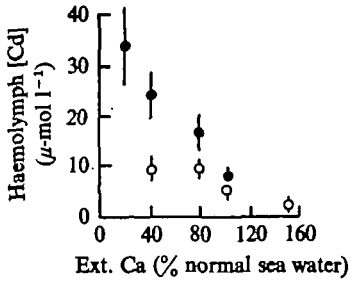


Fig. 1

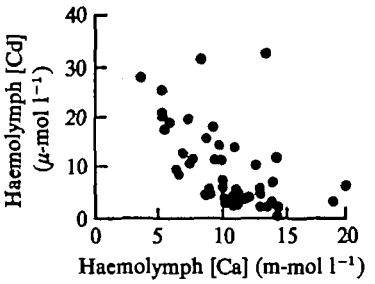
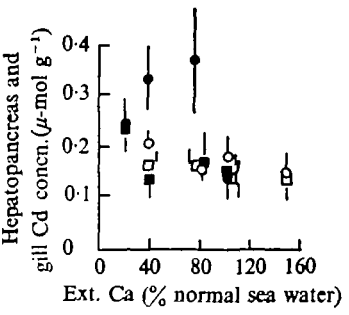


Fig. 2



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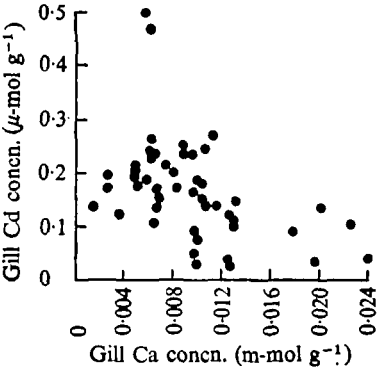

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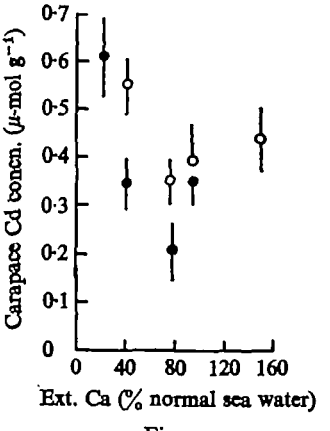

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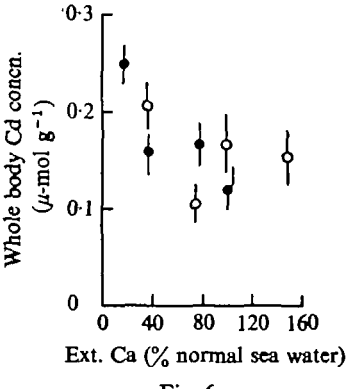

Fig. 6

Figs. 1, 3, 5, 6. Relationship between tissue Cd concentration and external (Ca). Solid symbols represent animals in 100% s.w. (except Ca). Open symbols represent animals in 40% s.w. (except Ca). Fig. 3. Hepatopancreas, circles; gill, squares. Vertical lines = S.E.'s. Figs. 2 and 4. Relationship between tissue Cd and Ca concentration for haemolymph and gills.

between these two parameters ($r = -0{\cdot}553$, $P < 0{\cdot}01$) which is apparently largely independent of the overall salinity of the external medium. These results are very interesting in the light of previous experiments (Wright, 1977*a*, *b*) which indicated that a high haemolymph cadmium concentration was associated with low external salinity. It is possible, then, that this so-called 'salinity effect' may simply be a function of the external calcium concentration and may be more accurately described

as a 'calcium effect'. One seemingly anomalous result requiring further mention is the significantly higher haemolymph cadmium concentration in 100% s.w., 40% Ca animals when compared with 40% s.w., 40% Ca animals. It is unfortunate in this respect that the latter were the only batch collected and studied at a different time from the other experimental animals. Nevertheless, although this has sometimes proved to be the source of some variability the differences here are quite marked and are probably real. Should this be the case the concept of a 'calcium effect' as mentioned above, would be reinforced. A further implication would be that a lower salinity (disregarding calcium) actually tends to *lower* the haemolymph cadmium concentration. One reservation which must be borne in mind however is that the relationship shown in Fig. 1 may be an indirect one, and that the haemolymph calcium:cadmium ratio may be more important. If the haemolymph calcium concentration is considered instead of the external calcium concentration a rather different picture emerges (Fig. 2). Here each animal has been considered separately and independently of overall salinity. The anomaly regarding animals exposed to 40% Ca largely disappears. This is due to the regulation of haemolymph calcium at a level above that of the external medium. Substituting haemolymph calcium concentration for external calcium thus has the effect of shifting the figures to the right. 40% s.w., 40% Ca animals were found to have a mean haemolymph calcium concentration of $8{\cdot}58 \pm 0{\cdot}92$ (S.E.) m-mol l^{-1}, and were thus shifted further to the right than 100% s.w. 40% Ca animals, which had a mean haemolymph calcium concentration of only $7{\cdot}08 \pm 0{\cdot}84$. These results are similar to those of Greenaway (1976) who found that *Carcinus* acclimated to dilute sea water were able to sustain a higher haemolymph calcium concentration than those acclimated to low calcium sea water. The relationship between haemolymph calcium and haemolymph cadmium (Fig. 2) therefore seems to be of a simple inverse nature ($r = -0{\cdot}355$, $P < 0{\cdot}05$).

The relationship between hepatopancreas and gill cadmium concentration and external calcium concentration is shown in Fig. 3. The correlation coefficients for hepatopancreas cadmium vs. external calcium and gill cadmium vs. external calcium are $-0{\cdot}28$ and $-0{\cdot}24$ respectively, neither of which reaches the 5% level of significance. The high hepatopancreas cadmium concentration in 100% s.w., 80% Ca animals results from a single exceptionally high value. No significant correlation could be found between hepatopancreas cadmium and calcium concentration. Gill cadmium and calcium, however, had a significant inverse relationship ($r = -0{\cdot}371$, $P < 0{\cdot}05$) (Fig. 4). Muscle cadmium concentration showed no significant relationship with either the external calcium concentration or the muscle calcium concentration and the data are not further considered here. The relationship between the carapace cadmium concentration and the calcium concentration of the external medium is shown in Fig. 5. The correlation between these two parameters was $-0{\cdot}24$, which failed to reach the 5% significance level. This was similar to the relationship between the carapace cadmium and calcium concentration where $r = -0{\cdot}20$. It is clear, however, that the carapace cadmium concentration strongly influenced the whole body cadmium level (Fig. 6), which did have a significant inverse correlation with the external calcium concentration ($r = -0{\cdot}35$, $P < 0{\cdot}05$). Whole body calcium concentrations were not available.

Therefore, it may be seen that after a period of exposure to cadmium at varying

Table 1. *Tissue Cd and Ca concentrations in animals at different stages in the moulting cycle after 6 days exposure to 20 μ-mol Cd l^{-1} in sea water*

(Tissue analyses on wet wt basis; •, see text.)

Animal no.	State	Haemolymph		Carapace		Hepatopancreas	
		Cd (μ-mol l^{-1})	Ca (m-mol l^{-1})	Cd (μ-mol g^{-1})	Ca (m-mol g^{-1})	Cd (μ-mol g^{-1})	Ca (m-mol g^{-1})
1•	Hard, premoult	22·8	17·7	0·27	4·24 (hard)	0·08	0·40
				0·04	0·13 (soft)		
2	V. soft, just postmoult	27·1	17·5	0·33	0·37	0·13	0·085
3	Soft, early postmoult	34·0	17·6	0·30	0·98	0·11	0·030
4	Leathery, late late postmoult	43·7	15·6	0·42	2·07	0·35	0·031
5	Leathery, late late postmoult	44·5	22·5	0·53	1·37	0·15	0·022
6	Leathery, late late postmoult	20·0	14·1	0·38	2·10	0·30	0·045
7	Leathery, late late postmoult	28·6	24·0	0·30	1·98	0·40	0·085
8	Hard, late postmoult	17·8	21·5	0·19	2·20	0·03	0·383
9	Hard, intermoult	15·5	10·1	0·13	4·43	0·31	0·046
10	Hard, intermoult	6·2	11·8	0·09	4·33	0·09	0·086
11	Hard, intermoult	4·8	11·4	0·08	4·41	0·10	0·230
12	Hard, intermoult	5·8	13·8	0·08	4·50	0·03	0·500
13	Hard, intermoult	6·8	14·0	0·13	5·90	0·03	0·511
14	Hard, intermoult	14·3	12·9	0·14	5·20	0·06	0·246

Animal no.	State	Gill		Muscle		Whole body	
		Cd (μ-mol g^{-1})	Ca (m-mol g^{-1})	Cd (μ-mol g^{-1})	Ca (m-mol g^{-1})	Cd (μ-mol g^{-1})	Ca (m-mol g^{-1})
1•	Hard, premoult	0·047	0·027	0·007	0·006	0·090	1·27
2	V. soft, just postmoult	0·037	0·010	0·063	0·017	0·110	0·046
3	Soft, early postmoult	0·054	0·009	0·104	0·019	0·139	0·355
4	Leathery, late late postmoult	0·076	0·016	0·081	0·039	0·127	0·374
5	Leathery, late late postmoult	0·078	0·011	0·150	0·038	0·148	0·384
6	Leathery, late late postmoult	0·070	0·015	0·075	0·009	0·128	0·348
7	Leathery, late late postmoult	0·117	0·009	0·061	0·026	0·131	0·319
8	Hard, late postmoult	0·059	0·013	0·005	0·011	0·070	1·240
9	Hard, intermoult	0·078	0·011	0·011	0·003	0·080	0·920
10	Hard, intermoult	0·013	0·017	0·006	0·010	0·048	0·861
11	Hard, intermoult	0·049	0·009	0·007	0·004	0·040	1·310
12	Hard, intermoult	0·027	0·013	0·008	0·003	0·035	1·100
13	Hard, intermoult	0·038	0·011	0·024	0·002	0·036	1·21
14	Hard, intermoult	0·037	0·021	0·049	0·021	0·051	1·76

Table 2. *Haemolymph calcium concentration in postmoult and intermoult animals in cadmium-free sea water*

Animal no.	State	Haemolymph calcium concentration (m-mol l^{-1})
15	Hard intermoult	13·4
16	Hard intermoult	12·8
17	Hard intermoult	12·3
18	Hard intermoult	13·8
19	Hard intermoult	12·2
20	Late postmoult, nearly hard	12·8
21	Late postmoult, nearly hard	10·0
22	Late postmoult, leathery	14·6
23	Late postmoult, leathery	12·5
24	Late postmoult, leathery	12·5
25	Postmoult, soft	9·0
26	Postmoult, soft	13·0
27	Postmoult, soft	9·8
28	Postmoult, soft	8·7
29	V. soft, within 3 h postmoult	13·0
30	V. soft, within 3 h postmoult	12·7

degrees of calcium depletion, a strong inverse correlation exists between calcium and cadmium in the haemolymph and gills of *Carcinus*. Data from other tissues are less convincing in this respect. However, considering the variability in the cadmium and calcium concentrations encountered within experimental batches, the varying degrees of calcium depletion attained, and the great difference between tissue calcium and cadmium concentrations (Ca:Cd ratio generally between 10^3 and 10^4), it was felt that the evidence was strong enough to merit further investigation using intermoult and postmoult crabs. It seemed likely that this would create a greater range of experimental material with regard to the calcium status of the animals.

Six soft postmoult crabs were used for this experiment. Eight hard crabs were used and although these were originally taken to be intermoult, one specimen had a fully formed soft exoskeleton beneath the outer hard one, and was therefore at the premoult stage. All animals were exposed to 100% s.w. + 20 μ-mol Cd l^{-1} for one week. After this period all animals were analysed for both calcium and cadmium. Five tissues including the haemolymph were analysed and figures were obtained for whole body calcium and cadmium in each case. The results are summarized in Table 1. Tissue calcium figures may be compared with earlier work by Robertson (1937). A number of interesting points emerge. The postmoult animals studied here (nos. 2–7) had a haemolymph calcium concentration of $18{\cdot}55 \pm 1{\cdot}59$ (S.E.) m-mol l^{-1}. The calcium concentration of the haemolymph of intermoult animals (9–14) was $12{\cdot}25 \pm 0{\cdot}64$ m-mol l^{-1}. These observations apparently differ from those of Robertson (1937, 1960) who noted a fall in haemolymph calcium in postmoult *Carcinus*. Haemolymph calcium in specimens between 2–14 days after moult was seen to fall to only 88% of the seawater calcium concentration, whereas haemolymph calcium in intermoult animals remained at 128% of the seawater calcium level (Robertson, 1960). A high haemolymph calcium concentration was usually found in premoult specimens, however, where levels exceeded 150% seawater calcium (Robertson, 1960). It was

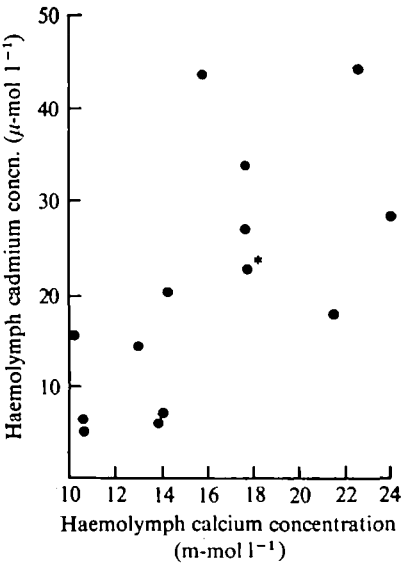

Fig. 7. The relationship between haemolymph cadmium and calcium in intermoult and postmoult *Carcinus*. Result from single late premoult animal also included (*). Correlation coefficient (r) for haemolymph Cd vs. haemolymph Ca = 0·63.

assumed that this transient rise was due to the withdrawal of calcium from the exoskeleton and that the fall in haemolymph calcium subsequent to the moult was a result of increased deposition of calcium in the skeleton. In view of the apparent contradiction between the results reported here and the earlier work it was necessary to obtain data from fresh postmoult specimens from cadmium-free sea water. As before, all collections of soft animals were made from the field and so it was impossible to be specific regarding the postmoult period. There were two exceptions to this, both of which were collected whilst in the process of moulting. All haemolymph analyses were made within 3 h of collection. The data are shown in Table 2 and largely agree with Robertson's findings. It is clear that the two freshly moulted animals were analysed before any significant depletion of haemolymph calcium concentration which was characteristic of Robertson's postmoult animals. Animals 20–24 were harder and had a haemolymph calcium concentration similar to intermoult control animals. It may be implied from these results and those in Table 1 that exposure to cadmium results in a raised haemolymph calcium concentration in postmoult *Carcinus*. This represents the only apparent alteration in ionic concentration noted during the present study and it is interesting to speculate as to the reason for this change. It may, at least in part, be due to some degree of blocking by cadmium of calcium deposition in the tissues of the postmoult animal. It is interesting that this results in a rise in haemolymph cadmium above the level normally found in intermoult animals. This in turn implies either a net release of calcium from the tissues of the postmoult animal or the action of an active calcium uptake mechanism possibly working at an increased rate under conditions where subsequent calcium deposition is inhibited. If such a mechanism is partially specific for cadmium then this might explain the nearly fourfold increase in haemolymph cadmium seen in postmoult animals (Table 1). A further explanation of the increase in both calcium and cadmium concentrations in the

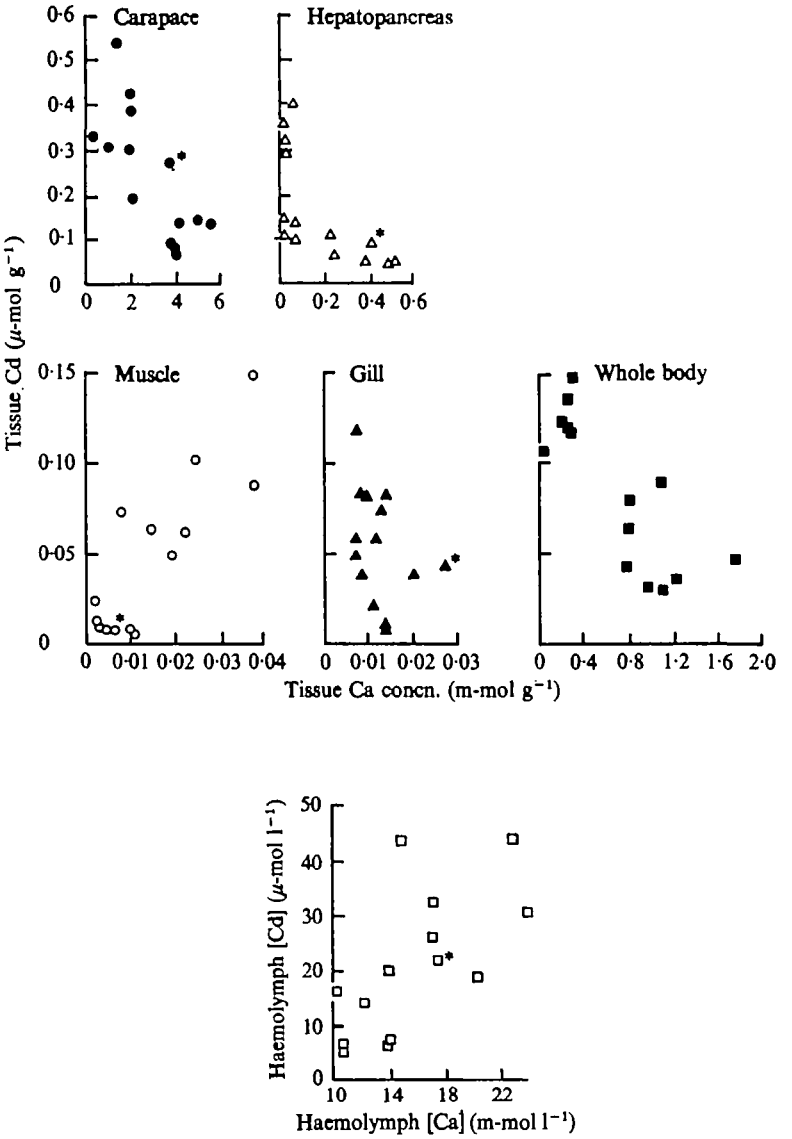

Fig. 8. The relationship between tissue and whole body cadmium and calcium concentration in intermoult and postmoult *Carcinus*. Results from a single late premoult animal are marked with an asterisk.

haemolymph of postmoult animals might be the incidence of increased haemolymph protein, thus creating more binding sites for both these metals. This is unlikely in view of Robertson's (1960) work which indicated a marked fall in haemolymph protein in postmoult animals. It is interesting then that here we have a converse situation to that found in calcium-depleted animals (Fig. 2). This is reflected in the highly significant correlation (r = 0·63) between haemolymph cadmium and calcium concentrations from animals described in Table 1, which is further illustrated in Fig. 7. The relationship between tissue cadmium and calcium for other tissues is shown in Fig. 8, where

the muscle is seen to follow the haemolymph in having a positive cadmium vs. calcium correlation ($r = 0{\cdot}80$). On the other hand there are highly significant inverse correlations between cadmium and calcium in the hepatopancreas and carapace ($r = -0{\cdot}68$ and $r = -0{\cdot}76$ respectively) and there is a similar correlation for the whole body cadmium/calcium data where $r = -0{\cdot}83$. No significant relationship was seen between gill cadmium and calcium in these animals ($r = 0{\cdot}251$). It is worth recording that, unlike intermoult animals, postmoult animals showed a slight increase in mortality when exposed to 20 μ-mol l^{-1} cadmium, although all results recorded here are from apparently healthy specimens.

Throughout this study determinations of tissue cadmium and calcium have been made on a wet weight basis. Although some factors have been supplied to enable conversion to dry weights (Wright, 1977*a*), these factors only apply to intermoult crabs. The work of Robertson (1937, 1960) shows that postmoult crabs may have a considerably higher water content than intermoult crabs (> 20% higher) and any such conversions for wet tissues of postmoult crabs would be under-estimates. The high cadmium levels noted in postmoult crabs would be further accentuated if their increased water content was taken into account.

DISCUSSION

The data presented here suggest that the accumulation of cadmium by the shore crab *Carcinus maenas* is to some extent dependent upon the calcium status of the animal.

In the haemolymph of intermoult animals the relationship between these two metals is a reciprocal one and it is tempting to suggest that this may be due to the progressive occupation by cadmium of haemolymph protein binding sites which under low calcium conditions are vacated by calcium. Greenaway (1976) has shown that under calcium-deficient conditions the bound haemolymph calcium fraction decreases roughly proportionately to the ionized fraction. A further possible explanation could be the stimulation of a calcium uptake mechanism under calcium deficient conditions. If such a mechanism also had some affinity for cadmium, then under conditions of maximum stimulation (calcium deficiency) cadmium uptake would increase. This latter explanation really amounts to 'accidental' active uptake and to some extent may be analogous to the relationship sometimes shared by sodium and lithium in transmembrane sodium transport (see Wright, 1975). However, it is probably safer to assume a rather more conservative approach at present and simply to suggest competition by calcium and cadmium for binding sites. The establishment of a similar relationship in other tissues has been less satisfactory although such a correlation is reinforced by data from moulting animals. Several studies have now established an inverse relationship between cadmium uptake by crustaceans and the salinity of the external medium (O'Hara, 1973*a*, *b*; Hutcheson, 1974; Wright, 1977*a*, *b*). The present work suggests that the concentration of the external calcium ion may be the most important single factor in this respect, and although some form of salinity effect *per se* is not necessarily ruled out in this or other species, it would be interesting to assess the relative importance of the calcium ion as a factor affecting cadmium accumulation by a wide range of aquatic animals.

The apparent rise in haemolymph calcium in postmoult animals exposed to cadmium is very interesting, particularly when compared with control animals, which were shown by Robertson (1937, 1960) to have a haemolymph calcium concentration lower than intermoult animals. This fall in haemolymph calcium concentration in postmoult animals in normal sea water has also been confirmed by current results. It seems then that here is evidence of an alteration in the normal ionic configuration of the haemolymph which has been caused by the presence of cadmium. Once again this may be explained in terms of competition between cadmium and calcium resulting in a blocking of the latter from prospective binding sites under conditions of maximum calcium uptake. This re-opens the question as to whether calcium uptake following the moult is an active or a passive process and would be a useful subject for further investigation.

It is clear from these data that the factors responsible for accumulation and distribution of cadmium within the body of *Carcinus* are apparently strongly influenced by the calcium metabolism of this animal. In view of the recent interest shown in cadmium accumulation by marine and estuarine species (Darracott & Watling, 1975; Eisler, Zaroogian & Hennekey, 1972; Fowler & Benayoun, 1974; Hutcheson, 1974; O'Hara, 1973*a*, *b*; Thurberg, Dawson & Collier, 1973; Westernhagen & Dethlefson, 1975; Westernhagen *et al.* 1974, 1975), the relationship between cadmium accumulation and calcium metabolism in such animals would seem to be a potentially useful line of research. Vertebral damage reported in fish exposed to cadmium (Bengtsson *et al.* 1975) may well be connected with this problem.

Studies such as this show that trace metal uptake by aquatic animals cannot always be considered in isolation from the processes involved in the accumulation and regulation of major ions.

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