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THE UPTAKE OF CADMIUM INTO THE HAEMOLYMPH OF THE SHORE CRAB *CARCINUS MAENAS*: THE RELATIONSHIP WITH COPPER AND OTHER DIVALENT CATIONS

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SUMMARY

When *Carcinus* was exposed to 20 μ-mol l^{-1} cadmium, the haemolymph cadmium level was initially dependent upon the salinity of the external medium. After 14 days the mean haemolymph cadmium level in 50% s.w. animals was nearly twice that of 100% s.w. animals. This trend was not sustained, however, and the situation was complicated by occasional inconsistent values.

In both *in vivo* and *in vitro* conditions nearly all the haemolymph cadmium becomes bound to haemolymph protein within a few days. The relationship between haemolymph cadmium, copper and protein concentration has been investigated. Although the latter are highly correlated with each other, cadmium formed a significant positive relationship with haemolymph copper ($r = 0{\cdot}523$) and protein ($r = 0{\cdot}533$) only after 3–4 weeks uptake. Exposure to 20 μ-mol l^{-1} cadmium has no obvious effects on haemolymph protein and copper concentrations, which are clearly dependent on feeding status. Mortalities among experimental animals were often preceded by a rise in haemolymph cadmium concentration. This is usually seen before there are any obvious signs of tissue breakdown.

Urine cadmium loss is probably unimportant as a pathway for the elimination of this metal. Urine cadmium concentrations often exceeded serum cadmium levels indicating that cadmium may sometimes be eliminated in bound form.

INTRODUCTION

It has been established that there is an inverse relationship between salinity and cadmium uptake by the gills and carapace of the shore crab *Carcinus maenas* (Wright, 1977). This relationship was reflected in the whole body cadmium concentration of the animal under different salinity regimes. The haemolymph was to some extent implicated in the 'salinity effect'.

In this paper haemolymph and urine cadmium analyses from 100% s.w. and 50% s.w. animals (Wright, 1977) are reported in detail, together with haemolymph protein, copper, calcium, and magnesium concentrations. If cadmium uptake is at least partially an active process, then an existing divalent cationic regulatory mechanism

may be involved. Copper occupies a special position in that, being part of the haemocyanin molecule, its haemolymph concentration gives a very good indication of the haemolymph protein level (largely haemocyanin). Bryan (1964, 1966) has found a positive relationship between haemolymph zinc, and haemolymph copper and protein in the lobster *Homarus vulgaris* and in *Carcinus*. In view of this, the degree of binding of cadmium to haemolymph protein is studied and the relationship between haemolymph cadmium, copper and protein noted. The investigation is extended to include animals sampled at intervals throughout a period of net cadmium uptake. Some attention is also paid to the relationship between bound and unbound haemolymph cadmium and the concomitant urine cadmium level.

MATERIALS AND METHODS

The animals and experimental procedure were as described previously (Wright, 1977).

Haemolymph samples were taken using a clean glass pipette inserted through the arthrodial membrane at the base of the third or fourth walking leg. To prevent the onset of clotting, cells were removed by centrifuging although this could also be accomplished by stirring the haemolymph with a thin glass rod. If the rod was twisted the cells adhered to it and could be drawn off from the sample. The cells represented between 0·6 and 1·2% by weight of the haemolymph and their removal made no noticeable difference to the levels of metals determined in this investigation.

Urine was obtained from the urinary pore on the coxopodite of the second antenna. Animals were clamped with their mouthparts upwards. After the surrounding area had been blotted dry with filter paper, the operculum covering the pore was lifted using a hooked needle and a fine pipette was placed immediately beneath it at the opening of the antennary gland. The pooled sample from both pores was generally between 200–500 μl. Urine samples obtained in this way are usually clear and uncontaminated with haemolymph. Any cloudy samples were discarded.

Most metal determinations were made using an EEL 240 atomic absorption spectrophotometer, although some of the smaller urine samples were analysed for cadmium using a Varian Techtron 1200 fitted with a carbon rod atomizer model 63. Correction for non-atomic absorption was by hydrogen continuum lamp operated at 228·8 nm. As before, when analysing calcium, 650 mg l^{-1} lanthanum chloride was used to offset interference.

The use of lanthanum chloride was extended to give a qualitative estimate of haemolymph protein levels. It was found that a haemolymph sample diluted 1:100 with distilled water gave a white precipitate on the addition of 50 μl of 6·5% w/v lanthanum as lanthanum chloride solution. Only gentle shaking was necessary to keep the precipitate in suspension for a matter of minutes, long enough for a turbimetric reading to be taken using a Gallenkamp CS-800 colorimeter (filter 520 μm but not critical). The density of the precipitate correlated very highly with haemolymph solids measured directly by drying and weighing, and with the haemolymph copper concentration. Readings therefore gave a quick, convenient estimate of the haemolymph protein status.

In view of the large numbers of haemolymph samples analysed, this method was

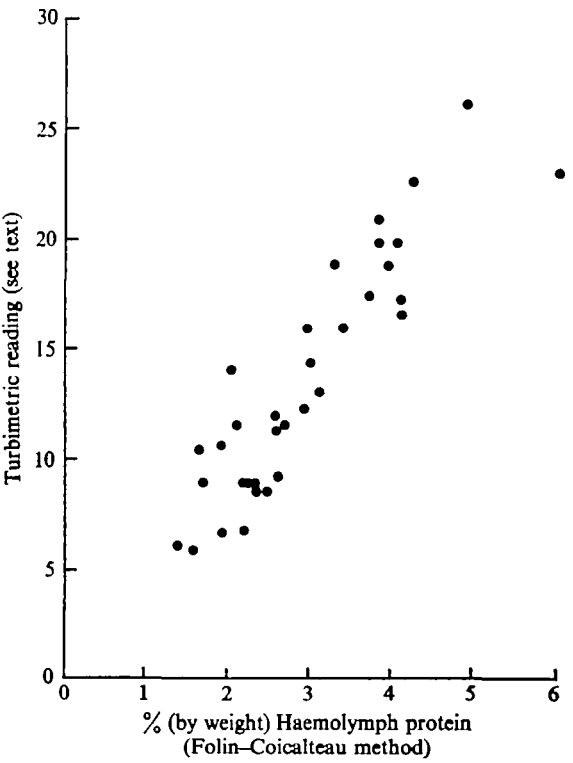

Fig. 1. The relationship between the lanthanum turbimetric method and the Folin–Coicalteau method for haemolymph protein determination. Samples from 35 animals.

preferred to a standard protein determination, although a preliminary comparison was made with the Folin–Coicalteau protein method (Fig. 1). The correlation coefficient between the two sets of analyses is 0·91, and from these the following relationship is obtained:

$$0{\cdot}44 + 0{\cdot}19 \times \text{turbimetric reading } (t) = \%\ \text{protein } (\pm 0{\cdot}48\%,$$

standard deviation calculated from scatter of the data).

The effect of filtering haemolymph samples to obtain protein-free serum was also investigated. Sartorius Cellulose-nitrate Membranfilters (SM 12136) were used, after soaking in sea water. The filters are 25 mm in diameter and have a pore size of 5 nm diameter, sufficient to remove proteins and other large molecules (MW 10000). The filters were placed in filter holder (SM 16251) and after adding 1–2 ml haemolymph an air pressure of $1{\cdot}05 \times 10^6$ kg m^{-2} was applied to the system. The filtrate was collected and analysed for metal content and these levels were compared with analyses of the unfiltered control.

RESULTS

For the first experiment the experimental groups (100% s.w. and 50% s.w.) were as described previously (Wright, 1977).

In both groups the cadmium concentration of the sea water was 20 μ-mol l^{-1}. At

Table 1. *Haemolymph copper and protein levels during net cadmium uptake from 100% s.w. + 20 μ-mol Cd l^{-1} and 50% s.w. + 20 μ-mol Cd l^{-1}*

(Figures represent mean ± S.E. (*n*). Number of days from start of experiment indicated; all animals starved throughout experimental period.)

	100% sea water			50% sea water		
Day	% by weight organic solids	Turbimetric reading	Copper (m-mol l^{-1})	% by weight organic solids	Turbimetric reading	Copper (m-mol l^{-1})
14	5·75 ± 0·62 (6)	20·52 ± 2·83 (6)	0·94 ± 0·09 (6)	3·83 ± 0·44 (6)	16·42 ± 1·60 (6)	1·12 ± 0·15 (6)
25	4·88 ± 0·99 (6)	19·27 ± 3·68 (6)	0·86 ± 0·13 (6)	3·78 ± 0·64 (6)	15·13 ± 3·56 (6)	0·84 ± 0·19 (6)
47	3·53 ± 0·71 (6)	12·63 ± 1·97 (6)	0·83 ± 0·14 (6)	3·03 ± 0·82 (4)	12·18 ± 4·11 (4)	0·69 ± 0·27 (4)
68	2·78 ± 0·46 (4)	10·40 ± 2·33 (4)	0·57 ± 0·14 (4)	2·23 ± 0·20 (3)	8·40 ± 1·91 (3)	0·49 ± 0·08 (3)

intervals over a period of 68 days haemolymph and urine samples were taken from batches of animals selected from each group.

Table 1 follows haemolymph copper and protein levels (turbimetric readings) during the period of net cadmium uptake. The latter were reinforced by direct weighings of haemolymph samples before and after evaporation. This procedure gave a measure of total haemolymph solids which could then be corrected, taking into account the inorganic salts present, to give a percentage by weight of solid organic material, largely protein. From Fig. 2 it may be seen that these two methods of haemolymph protein estimation compare well. Furthermore, the copper concentration correlates highly with haemolymph protein (for copper vs. protein $r = 0{\cdot}84$). There are indications from the data (Table 1) of a fall in haemolymph copper and protein levels throughout the experimental period.

Cadmium levels in haemolymph and dialysed serum (Table 2) indicate that there is progressive binding of free cadmium throughout the experimental period until the bound portion represents virtually all of the total haemolymph cadmium. It is interesting in this respect that some urine cadmium levels apparently occupy an intermediate position between the total haemolymph and protein-free serum resulting in a higher mean for urine Cd than for serum Cd. This could possibly be explained by the presence of a haemolymph fraction capable of binding cadmium, which despite having a large molecular weight is still cleared by the antennary gland. Mean figures for 100% s.w. animals after 68 days (Table 2) are considerably influenced by a single animal (No. 19 – see later) which had an exceptionally high haemolymph cadmium concentration of 27·8 μ-moles (serum Cd 0·8 μ-mol l^{-1}, urine Cd 0·5 μ-mol l^{-1}).

Two aspects of this work were further investigated. The aims were:

(1) To ascertain whether the binding of cadmium to haemolymph protein was a passive process which would occur *in vitro*.

(2) To investigate the possible presence of bound cadmium in the *urine*, which would account for the occasional discrepancies between urine cadmium and (filtered) serum cadmium.

Four 5 ml samples were made up from the haemolymph of 12 fresh crabs, after

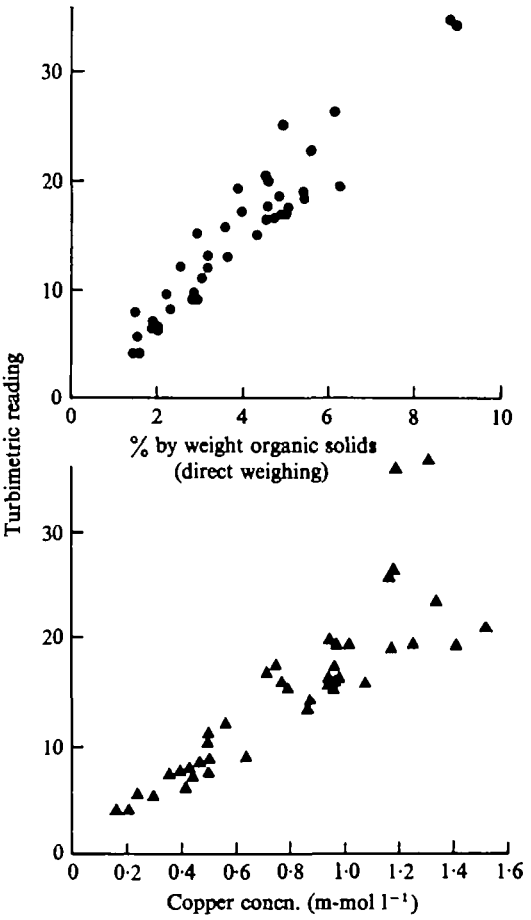

Fig. 2. Relationship between haemolymph protein (measured turbimetrically), % by weight organic solids and copper concentration during net cadmium uptake (animals 1–41).

first removing the haemolymph cells to prevent clotting. 0·2 ml of 200 μ-moles cadmium chloride (in crab Ringer) was added to each sample making a final haemolymph concentration of 8 μ-mol l^{-1} cadmium, a level within the range found in experimental animals during net cadmium uptake. After checking the initial cadmium concentration the samples were then stored in stoppered glass bottles in a refrigerator (2–4 °C). At intervals of 24 h, 48 h, 1 week and 2 weeks, aliquots of approximately 1 ml were taken from each sample and split into two parts, one for immediate measurement of cadmium concentration and the other for filtration through a cellulose nitrate filter followed by cadmium analysis of the filtrate (serum). These measurements showed the relative proportions of bound to unbound cadmium present and gave an indication of the time course of the progressive binding of cadmium to haemolymph proteins in the *in vitro* state (Fig. 3).

The picture of cadmium incorporation into haemolymph protein is somewhat similar to the situation in the intact animal during net cadmium uptake where almost all the haemolymph cadmium is present in a bound form after the first few days of net

Table 2. *Haemolymph and urine cadmium levels (in μ-mol l^{-1}) during net cadmium uptake from 100% s.w. + 20 μ-mol Cd l^{-1} and 50% s.w. + 20 μ-mol Cd l^{-1}*

(Figures represent mean ± S.E. (*n*). Same animals as in Table 1.)

	100% sea water			50% sea water		
Day	Haemolymph Cd	Serum Cd	Urine Cd	Haemolymph Cd	Serum Cd	Urine Cd
14	3·38 ± 0·96 (6)	0·58 ± 0·17 (6)	0·60 ± 0·24 (6)	5·65 ± 0·62 (6)	0·47 ± 0·19 (6)	0·93 ± 0·18 (6)
25	6·38 ± 0·88 (6)	0·27 ± 0·17 (6)	0·78 ± 0·29 (6)	5·82 ± 1·07 (6)	0·17 ± 0·08 (6)	0·58 ± 0·11 (6)
47	4·97 ± 0·83 (6)	0·17 ± 0·10 (6)	0·73 ± 0·39 (6)	8·68 ± 1·76 (4)	0·13 ± 0·08 (4)	2·03 ± 1·04 (4)
68	10·53 ± 5·82 (4)	0·28 ± 0·19 (4)	0·48 ± 0·21 (4)	5·67 ± 2·01 (3)	0·10 ± 0·10 (3)	0·27 ± 0·18 (3)

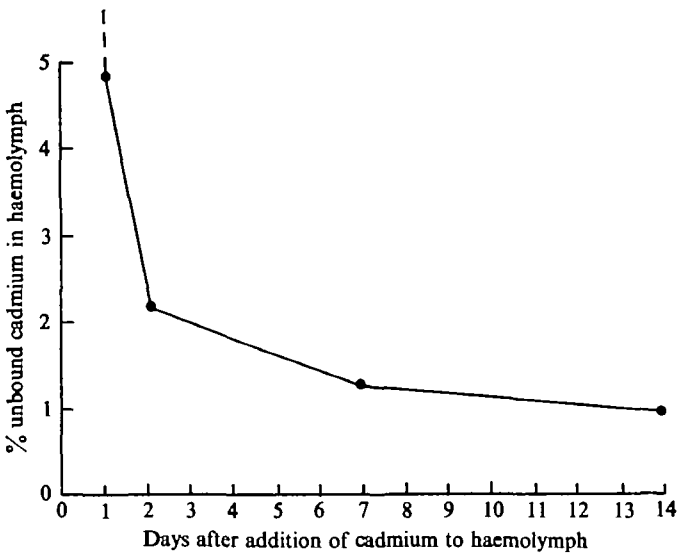

Fig. 3. The time course of binding of cadmium to haemolymph protein *in vitro*.

uptake. Taken together, the results indicate that at least at the onset of cadmium uptake the cadmium is passively bound to proteins already present in the haemolymph rather than being actively incorporated into newly constituted protein. The presence of some unbound cadmium in the haemolymph after 5 days of uptake indicates that the attachment of cadmium to the haemolymph protein is not an immediate process. Nevertheless, it is interesting to note that after the disappearance of this unbound cadmium *in vivo*, the free cadmium fraction remains at a very low level even though net cadmium uptake apparently continues. This indicates that some form of equilibrium is reached, whereby any cadmium reaching the haemolymph subsequently is quickly bound to the protein in the haemolymph. An alternative would be the rapid passage of free cadmium through the haemolymph to the tissues 'by-passing' the bound fraction. The rate of turnover of this bound cadmium must await further study.

Table 3. *Cadmium analyses (μ-mol l^{-1}) of pooled urine and haemolymph samples before and after dialysis*

Sample no.* and experimental medium cadmium concn.	Whole haemolymph cadmium concn.	Filtered serum cadmium concn.	Urine cadmium concentration	Filtered urine cadmium concn.
(i) 20 μ-mol l^{-1}	3·00	1·80	1·81	1·90
(ii) 20 μ-mol l^{-1}	5·81	1·62	1·90	1·88
(iii) 20 μ-mol l^{-1}	5·78	0·20	0·23	0·20
(iv) 50 μ-mol l^{-1}	11·10	2·47	5·75	3·80

* Each sample is pooled from 6–7 animals.

Table 4. *Haemolymph calcium and magnesium levels (in m-mol l^{-1}) during net cadmium uptake from* 100% *s.w.* + 20 μ-*mol Cd* l^{-1} *and* 50% *s.w.* + 20 μ-*mol Cd* l^{-1}

(Figures represent mean ± S.E. (*n*). Same animals as in Tables 1 and 2.)

Day	Haemolymph Ca	Serum Ca	Haemolymph Mg	Serum Mg
		100% sea water		
14	13·87 ± 0·72 (6)	10·88 ± 0·51 (6)	22·80 ± 0·93 (6)	22·45 ± 0·81 (6)
25	13·95 ± 0·73 (6)	11·9 ± 0·81 (6)	20·80 ± 1·46 (6)	20·95 ± 2·45 (6)
47	13·02 ± 0·34 (6)	9·60 ± 1·00 (6)	22·18 ± 1·29 (6)	22·08 ± 2·24 (5)
68	12·28 ± 0·56 (4)	—	19·78 ± 1·18 (4)	—
		50% sea water		
14	9·05 ± 0·54 (6)	7·10 ± 0·98 (6)	10·30 ± 0·67 (6)	10·08 ± 0·89 (6)
25	9·58 ± 0·55 (6)	7·77 ± 0·66 (6)	10·62 ± 0·61 (6)	10·44 ± 0·59 (6)
47	8·60 ± 0·33 (4)	7·23 ± 0·58 (4)	12·20 ± 1·81 (4)	10·75 ± 0·99 (4)
68	8·63 ± 0·19 (3)	—	9·75 ± 1·15	—

The relationship between bound and unbound cadmium in the haemolymph and urine was studied in crabs which had all been exposed to 20 μ-mol l^{-1} or 50 μ-mol l^{-1} cadmium for 2 weeks. In order to study the possible presence of bound cadmium in the urine it was necessary to pool urine samples from six or seven animals. It was not always possible to obtain a standard volume of urine from these animals. Volumes of urine from individual animals therefore ranged between 100 and 300 μl. Haemolymph samples were also taken from each animal and pooled samples were constituted which corresponded proportionally to the pooled urine samples. For example, if the urine volume from one animal was twice that of a second animal, then its contribution to the pooled haemolymph sample was also double that of the second animal. Four pooled urine samples with corresponding haemolymph samples were obtained in this way. Each pooled sample was split into two parts, for cadmium analysis before and after filtration. The results are shown in Table 3. In three groups there was no apparent difference between filtered and unfiltered urine cadmium, indicating an absence of

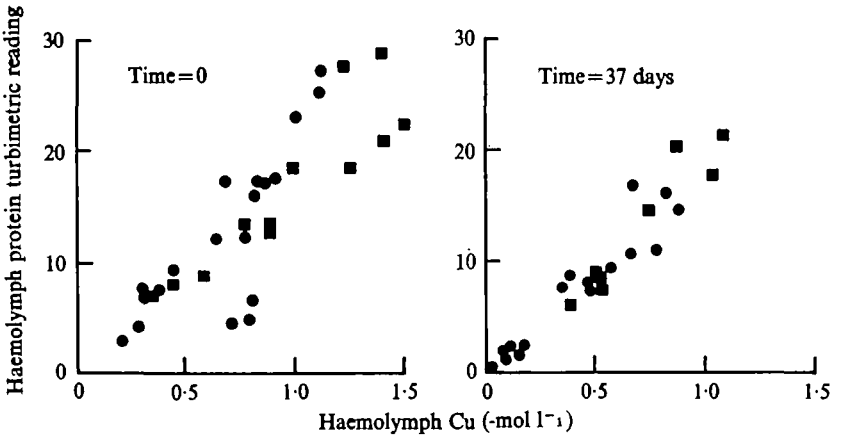

Fig. 4. Haemolymph protein/copper correlation in single batch of animals before and after 37 days cadmium uptake under starvation conditions.

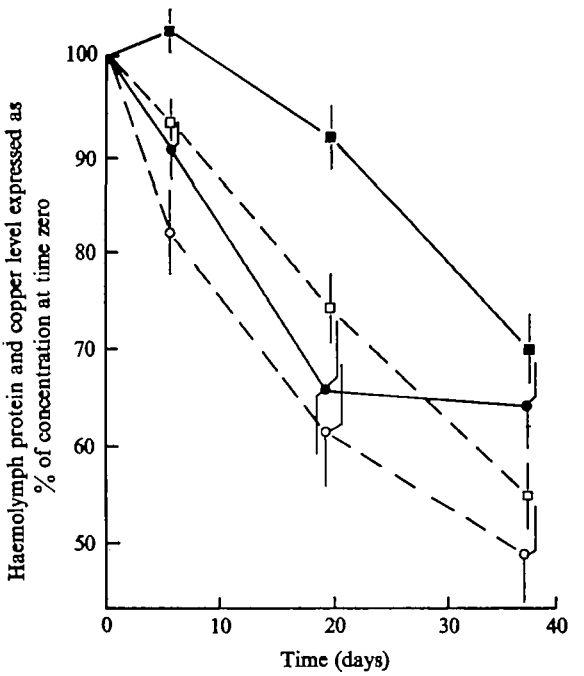

Fig. 5. Haemolymph copper and protein level during 37 day Cd uptake period (same animals as in Fig. 4). ■, Cu, fresh animals; □, Cu, animals semi-starved before experiment; ●, protein, fresh animals; ○, protein, animals semi-starved before experiment.

bound cadmium. However, the fourth group, albeit exposed to a higher external cadmium than the others, did show a significant proportion of urine cadmium which was non-dialysable. Such a result could explain the occasional urine cadmium level that was higher than the corresponding filtered haemolymph cadmium concentration.

Table 4 shows calcium and magnesium concentrations in the whole and dialysed haemolymph of experimental animals. Although in neither case was there any significant correlation with either haemolymph protein or cadmium ($0{\cdot}05 < P < 0{\cdot}1$), the results have been included for comparison with other published data. Such comparison reveals no apparent abnormalities in the calcium and magnesium concentrations reported here. For example, the data are comparable with figures given by Shaw (1955) and Greenaway (1976) for *Carcinus*. Moreover, there is good agreement between the current data and Greenaway's figures for total and ionized calcium in *Carcinus* haemolymph, obtained using an ion-specific electrode.

Table 2 reveals no consistent relationship between the haemolymph cadmium concentration of the two groups. Although the 50% s.w. group appears to have a generally higher haemolymph cadmium concentration than the 100% s.w. group after 14 days (see also Wright, 1977), this trend is not sustained and the situation is complicated by occasional inconsistent values.

In order to investigate the relationship between haemolymph cadmium and haemolymph protein, it was therefore decided, because of the considerable individual variability, to take a series of samples from the same group of animals during net cadmium uptake. In order to have experimental animals of varying haemolymph protein status, three different batches were used. One batch (animals nos. 42–47) was kept in a semi-starved condition in laboratory aquaria for 3 weeks prior to the experiment (animals may be kept in this state for several months without deleterious effects). A second batch (nos. 48–54) were also semi-starved but were force-fed with *Mytilus* gill 24 h before the commencement of the experiment. Despite this, no clear differences between these two batches emerged and these animals are hereafter referred to as semi-starved. Another batch (nos. 55–60) was comprised of fresh animals collected within 24 h of experimentation. Two control batches (kept in 'cadmium-free' sea water) were used in the experiment to expose any changes in haemolymph protein level caused directly by the presence of cadmium. The control batches comprised semi-starved (nos. 61–66) and fed animals (67–72). Before the experiment, haemolymph samples were taken from each animal and subjected to copper and protein (turbimetric) analysis. In view of the possible cadmium/calcium relationship suggested by the high shell cadmium concentrations in a previous experiment (Wright, 1977), haemolymph calcium determinations were made in both experimental and control animals. Experimental animals were then placed in 20 μ-mol l^{-1} cadmium chloride in sea water and further haemolymph samples were taken after periods of 5, 22 and 37 days. These samples were analysed for copper, cadmium, and protein. The relationship between haemolymph protein and copper was followed throughout the experimental period. The correlation coefficient (r) between these parameters at the beginning of the experiment was 0·92; day 5, $r = 0{\cdot}92$; day 22, $r = 0{\cdot}94$; day 37, $r = 0{\cdot}96$. Although the correlation apparently improves throughout this period, the correlation coefficients are not directly comparable, owing to the fall in number of animals due to mortalities. The relationship between haemolymph protein and copper

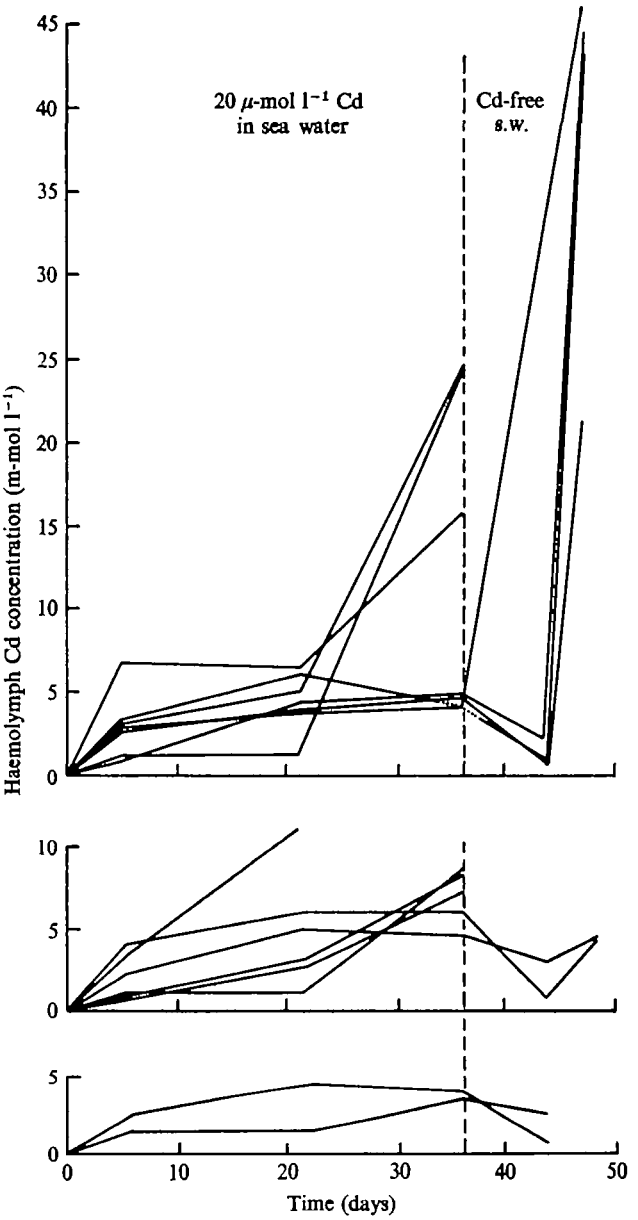

Fig. 6. Time course of cadmium uptake into the haemolymph of individual animals (discontinued lines represent animals killed or died).

at the beginning of the experiment and on day 37 is shown in Fig. 4. All animals were kept under starving conditions throughout the 37-day period of net uptake, and it is of interest to follow the fall in haemolymph protein and copper during this time (Fig. 5). Fresh animals are capable of maintaining or even raising their haemolymph copper level over a period of at least 5 days after collection. After this period a significant fall is apparent, which is sustained at about 1·3% original haemolymph

Table 5. *Haemolymph analyses and description of animals having an abnormally high haemolymph cadmium concentration*

Animal no.	Day when sample taken	State of animal	Haemolymph Cd (μ-mol l^{-1})	Filtered haemolymph Cd (μ-mol l^{-1})	Haemolymph protein (turbimetric reading)	Haemolymph Cu (m-mol l^{-1})
19	68	apparently healthy	27·8	0·8	17·3	1·0
42	37	unhealthy	24·9	0·9	6·9	0·35
48	37	apparently healthy	25·0	3·4	1·4	0·13
58	37	apparently healthy	16·1	1·7	8·0	0·42
43	44	unhealthy	45·8	2·1	1·8	0·06
49	47	recently dead	44·5	—	11·0*	0·58
55	47	recently dead	43·3	—	18·6*	0·64
56	47	apparently healthy	20·8	—	6·9	0·49

Dashes indicate no analysis made.

* Figures represent significant rises in haemolymph protein since previous analysis.

copper concentration per day. On the other hand a significant fall in total haemolymph protein in fresh animals is recorded after 5 days and continues at a rate of about 1·4% original haemolymph protein per day for about 3 weeks. After this period there is a reduction in rate of disappearance of protein from the haemolymph. For both copper and protein these trends are continued in semi-starved animals. It seems clear that, under conditions of starvation, the haemolymph protein is initially depleted at the expense of the non-copper protein fraction but subsequently there is a steady reduction in the copper-containing fraction. Such a sequence would result in an increased correlation between haemolymph copper and protein and may at least partially explain the results shown in Fig. 4.

As cadmium had no apparent direct effect on either the protein or copper concentration of the haemolymph, the results described above were obtained from both experimental and control animals. Cadmium uptake into the haemolymph of experimental animals is shown in Fig. 6, where the variability of the data necessitates the presentation of individual results. As mortality in both experimental and control animals rose sharply after 37 days, further detailed study of cadmium uptake was impossible. Some of the remaining animals were then returned to 'cadmium-free' sea water for an investigation of cadmium loss from the haemolymph, although mortality again curtailed this. The death of experimental animals was often associated with a sudden rise in the haemolymph cadmium level, even when animals had been in cadmium-free sea water for several days. Animals showing a sudden rise in haemolymph cadmium are further considered in Table 5, along with a specimen, no. 19, from the first experiment. That this rise in haemolymph cadmium is indicative of imminent death is well illustrated by specimens 42, 48 and 58. Animal no. 42 was clearly unhealthy at the time of sampling on day 37, although the other two animals appeared perfectly healthy. However, all were dead within 24 h. Dialysis of the haemolymph soon after taking the sample revealed the cadmium to be mainly in a bound form, as with specimens 19 (first experiment) and 49. Furthermore, it is interesting that despite the elevated haemolymph cadmium levels in animals 42, 48 and 58, *no* indication of increased haemolymph protein is given by the corresponding turbimetric readings. This suggests that the cadmium which floods into the haemo-

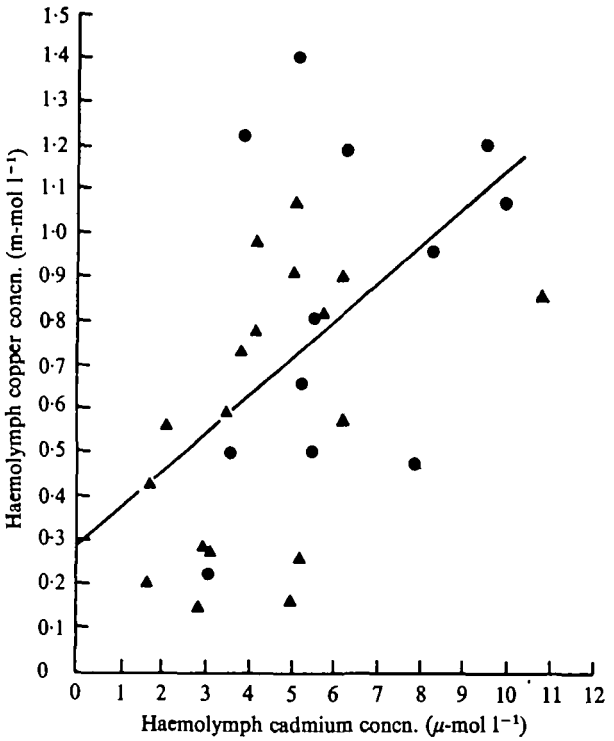

Fig. 7. The relationship between haemolymph cadmium and copper concentration after a period of 22/25 days cadmium uptake. ●, 50% s.w. and 100% s.w. animals, 1st experiment, 25 days; ▲, 100% s.w. animals, 2nd experiment, 22 days.

lymph close to the death point is initially unbound but it is subsequently incorporated into existing haemolymph protein. Results from animals 43 and 49 indicate that it is only after death that excess protein is released into the haemolymph, presumably from tissue breakdown. Ignoring the abnormally high values there is evidence from the present data that an upper limit for haemolymph cadmium of 5–8 μ-mol l^{-1} is reached in healthy animals after about 30 days in 20 μ-mol l^{-1} cadmium although the variability of the data must again be emphasized. In some animals a fall in haemolymph cadmium was noted between successive analyses.

The haemolymph concentrations of cadmium and copper only correlate significantly after about 3 weeks of net cadmium uptake. In Fig. 7 the results from 100% s.w. and 50% s.w. animals after 25 days exposure to 20 μ-mol l^{-1} cadmium (7–12, 29–34) have been pooled with animals 42–60 having had 22 days in 20 μ-mol l^{-1} cadmium. The correlation coefficient is 0·523 which is significant ($P < 0{\cdot}01$). The correlation coefficient between haemolymph cadmium and protein in these animals is 0·533 ($P < 0{\cdot}01$) (Fig. 8).

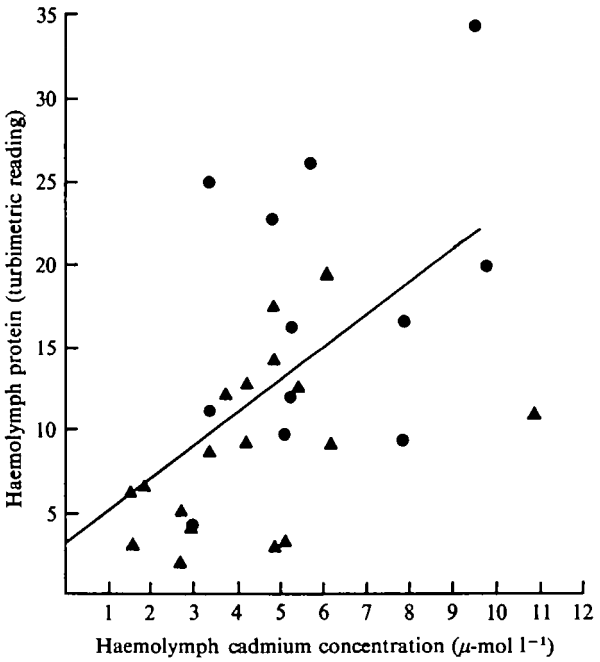

Fig. 8. The relationship between haemolymph cadmium and protein concentration after a period of 22/25 days cadmium uptake. ●, 50% s.w. and 100% s.w. animals, 1st experiment, 25 days; ▲, 100% s.w. animals, 2nd experiment, 22 days.

DISCUSSION

It is clear that there is not such an obvious salinity effect on cadmium in the haemolymph of *Carcinus* as is found in some other tissues (Wright, 1977). Results from the first experiment do indicate a faster cadmium uptake by 50% s.w. animals over the first 2 weeks (see also Wright, 1977), although this effect subsequently disappears.

The haemolymph cadmium concentration apparently reaches an equilibrium value less than half that of the external medium, nearly all of it becoming bound to the haemolymph protein fraction. Bryan (1971) suggests that the absorption of zinc by crustaceans could be by a process of diffusion from water to haemolymph following adsorption of the metal onto the cuticle of (say) the gills. He postulates that the tight binding of the metal to haemocyanin in the haemolymph would be sufficient to create a concentration gradient for unbound zinc from water to haemolymph. It is conceivable that here, too, the seawater/haemolymph concentration gradient may favour the movement of free cadmium into the haemolymph. However, there are still a number of questions which must be answered before we can be certain about the nature of cadmium accumulation by this animal. An immediate problem confronting the theory of passive absorption of heavy metals with a strong affinity for protein concerns the liberation of the metal into the haemolymph from its association with gill proteins. Whether or not such proteins are 'carrier' molecules under active control is also open to question (O'Hara, 1973; Wright 1977). If the concentration gradient is sufficient to effect the release of metal from the gill, then we must assume that any such metal

entering the haemolymph immediately becomes associated with haemolymph proteins. Of further interest is the rate of turnover of this bound cadmium and its release to tissues for storage and excretion. The diminution of haemolymph cadmium in many animals transferred to cadmium-free sea water suggests that this turnover of cadmium may be fairly rapid, although this is currently under investigation.

The relationship between haemolymph cadmium and copper/protein after about 3 weeks of cadmium uptake is rather similar to the relationship between zinc and copper (and protein measured as % solid content) in the haemolymph of *Carcinus* exposed to 100–115 μg l^{-1} (= 1·7 μ-mol l^{-1}) zinc in sea water (Bryan, 1966). The correlation between haemolymph cadmium and copper/protein is weaker than that found for zinc and it is interesting to note that Bryan (1967) found no zinc copper correlation in the freshwater crayfish *Austropotamobius pallipes* where starch-gel electrophoresis revealed the zinc to be bound to non-copper protein. A similar procedure would be needed to clarify the position of bound cadmium in the haemolymph of *Carcinus*. The relationship between haemolymph copper and protein found in the present study is nearly identical to that reported for *Carcinus* by Bryan (1966). It was clear that the steady fall in haemolymph copper and protein concentration noted throughout the experimental period was not associated with a concomitant fall in haemolymph cadmium level. This could be explained by the relocation of cadmium from decomposed protein on remaining haemolymph protein, or the supplementation of the potentially decreasing cadmium concentration by uptake from the external medium. The rate of diminution of haemolymph protein found in this investigation is similar to, although a little faster than, that reported for starved specimens of *Carcinus* by Uglow (1969). He found that total haemolymph protein fell at a mean rate of approximately 1 % per day over a 28-day period and that both copper-containing and non-copper-containing protein were involved. In fed animals no significant decrease in haemolymph protein was noted. The fall in haemolymph copper and protein observed in the present study was probably accelerated by the taking of haemolymph samples. Horn & Kerr (1963) reported that serial bleeding of the blue crab *Callinectes sapidus* resulted in a fall in total haemolymph protein. These authors also noted an increase in the copper:protein ratio. Although a similar phenomenon is seen here with fed animals over the first 3 weeks, there is insufficient evidence to attribute this solely to the taking of haemolymph samples.

Bryan (1966) found that the urine zinc level in *Carcinus* was strongly correlated with the serum zinc concentration. He concluded that the latter controlled haemolymph zinc regulation and that its appearance in the urine was the result of a process of simple filtration. It seems possible that this cadmium may be in bound form, although this requires further study. Small, Keckes & Fowler (1974) suggested that zinc may be excreted from the prawn *Palaemon serratus* in either unbound or complex form, although they were unspecific about the nature of the complexed zinc. Bryan (1966) considered that, in the excretion of zinc by *Carcinus*, the antennary gland was secondary to the gills. For example, he found that if extra zinc was injected into the haemolymph, about 85 % of this was subsequently eliminated via the gills. With cadmium, too, the antennary gland is unlikely to be an important excretory route in *Carcinus*. If we assume the cadmium loss from an animal to be approximately 0·08 μ-mol g^{-1} over 11 days (Table 4), and take the urine flow rate to be 4 % body weight day^{-1}

(unpublished observations) a urine cadmium concentration of about 180 μ-mol l^{-1} would be necessary to sustain such a loss through the antennary gland alone. Investigating zinc loss from decapod crustaceans, Bryan (1964, 1966) found urine zinc excretion to be important in *Homarus*, although in *Carcinus* the gills were the major site of zinc elimination.

REFERENCES

BRYAN, G. W. (1964). Zinc regulation in the lobster, *Homarus vulgaris*. I. Tissue zinc and copper concentrations. *J. mar. biol. Ass. U.K.* **44**, 549–63.

BRYAN, G. W. (1966). The metabolism of Zn and ^{65}Zn in crabs, lobsters and freshwater crayfish. *Symp. Radioecological Concentration Processes, Stockholm, Sweden*, pp. 1005–16. Oxford: Pergamon Press.

BRYAN, G. W. (1967). Zinc regulation in the freshwater crayfish (including some comparative copper analyses). *J. exp. Biol.* **46**, 281–96.

BRYAN, G. W. (1971). The effects of heavy metals (other than mercury) on marine estuarine organisms. *Proc. R. Soc.* B **177**, 389–410.

GREENAWAY, P. (1976). Regulation of haemolymph calcium concentration of the crab *Carcinus maenas* (L.). *J. exp. Biol.* **64**, 149–57.

HORN, E. C. & KERR, M. S. (1963). Haemolymph protein and copper concentrations of adult blue crabs (*Callinectes sapidus* Rathburn). *Biol. Bull. mar. biol. Lab., Woods Hole* **125**, 499–507.

O'HARA, J. (1973). Cadmium uptake by fiddler crabs exposed to temperature and salinity stress. *J. Fish. Res. Bd Can.* **30**, 846–8.

SHAW, J. (1955). Ionic regulation in the muscle fibres of *Carcinus maenas*. II. The effect of reduced blood concentration. *J. exp. Biol.* **32**, 664–80.

SMALL, L. F., KECKES, S. & FOWLER, S. W. (1974). Excretion of different forms of zinc by the prawn *Palaemon serratus* (Pennant). *Limnol. Oceanogr.* **19**, 789–93.

UGLOW, R. F. (1969). Haemolymph protein concentrations in portunid crabs. II. The effects of imposed fasting on *Carcinus maenas*. *Comp. Biochem. Physiol.* **31**, 959–67.

WRIGHT, D. A. (1977). The effect of salinity on cadmium uptake by the tissues of the shore crab, *Carcinus maenas* (L.). *J. exp. Biol.* **67**, 137–46.