Effects of cadmium on hemolymph composition in the shore crab *Carcinus maenas*

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ABSTRACT: Effects of cadmium exposure on regulation of osmolality and sodium, chloride, potassium, calcium, magnesium, and cadmium levels in the hemolymph of the shore crab *Carcinus maenas* (L.) were studied. Exposure to 1 mg Cd l^{-1} at 15.5 °C and 400 mOsm (~14 ‰) had no effects on ionic concentrations during 48 d. Exposure to ≥ 4 mg Cd l^{-1} augmented calcium levels in the hemolymph, while magnesium concentrations were reduced after prolonged exposure to 2 and 4 mg Cd l^{-1}. In some experiments exposure to 10 mg Cd l^{-1} augmented Na^+, K^+, and Cl^- concentrations and osmolality, but the effects were not consistent. Crabs exposed to ≤ 2 mg Cd l^{-1} maintained cadmium levels in the hemolymph at or slightly below ambient cadmium concentrations, while cadmium levels in the hemolymph rose above ambient levels in crabs exposed to 4 to 10 mg Cd l^{-1}. Effects on calcium levels in the hemolymph coincided with increases in cadmium levels in the hemolymph. More than 95 % of the cadmium in the hemolymph was bound to high molecular weight proteins and upon *in vitro* addition of cadmium to hemolymph samples free cadmium could not be detected before the cadmium concentration exceeded 16 µg Cd ml^{-1}.

INTRODUCTION

Cadmium contamination of the marine environment due to increased anthropogenic input is most likely to occur in coastal and estuarine areas. Organisms inhabiting these areas encounter wide variations in external salinities and ion concentrations. Reduction in salinity augments uptake rate and toxicity of cadmium in brachyuran crabs (O'Hara 1973a, b, Hutcheson 1974, Rosenberg & Costlow 1976, Wright 1977a, b) and other marine organisms (reviewed by Coombs 1979).

Cadmium uptake and accumulation in the shore crab *Carcinus maenas* has been studied in great detail in recent years (Wright 1977a, b, c, Jennings & Rainbow 1979, Jennings et al. 1979, Rainbow & Scott 1979, Wright & Brewer 1979, Bjerregaard 1982, Chassard-Bouchaud 1982). Although the effect of reduced salinity on cadmium toxicity is well established (Coombs 1979), investigations of cadmium's effect on ion- and osmoregulation processes in *C. maenas* and other marine organisms are scarce.

At salinities below 33 ‰ *Carcinus maenas* normally maintains hemolymph osmolality and Na^+, Cl^-, K^+, and Ca^{++} levels above those of the ambient seawater (Webb 1940, Robertson 1960, Shaw 1961, Theede 1969, Greenaway 1976, Zanders 1980a), while Mg^{++} concentrations are held substantially below ambient sea-

water concentrations in 15 to 150 % seawater (Lockwood & Riegel 1969, Zanders 1980b). Thurberg et al. (1973) reported an augmenting effect of cadmium on total osmolality in the hemolymph of *C. maenas*, but the effect of cadmium on regulation of individual inorganic hemolymph ions has not been investigated.

Wright (1977a, b, c) and Wright & Brewer (1979) observed that the cadmium concentration in the hemolymph of *Carcinus maenas* remains at a lower level than that of the surrounding medium, even if the latter is close to the background concentration or raised to 2.4 mg Cd l^{-1}. Wright & Brewer (1979) suggest that the cadmium concentration of the hemolymph can be kept at a low level because cadmium after uptake via the gills is removed from the hemolymph by uptake in the hepatopancreas. Jennings et al. (1979) suggest that cadmium is transported in the hemolymph, bound to specific low molecular weight proteins with a high affinity for cadmium. Chassard-Bouchaud (1982) suggests that *C. maenas* detoxifies cadmium in the hepatopancreas by immobilizing the metal in mineral concretions. It is not known if *C. maenas* can regulate hemolymph cadmium levels at ambient cadmium concentrations above 2.4 mg Cd l^{-1} and if breakdown of this regulatory ability will lead to toxic manifestations in the crab.

We have therefore conducted a series of experiments

to examine the effect of cadmium on ion- and osmo-regulatory processes in *Carcinus maenas*. We also wanted to investigate if the effects of cadmium on physiological processes could be attributed to accumulation and binding pattern of cadmium in the hemolymph.

MATERIALS AND METHODS

Adult male shore crabs *Carcinus maenas* (L.) were caught in seines in Little Belt, Denmark. The prehistory (concerning salinity) of the crabs was unknown since the salinity of Little Belt water may vary between 14 and 30 ‰, depending on depth and prevailing wind and stream (L. P. D. Rasmussen, pers. comm. 1985). Crabs used in February to April were caught in October and maintained in flowing seawater aquaria at the marine biological station Bøgebjerg, N. E. Funen, Denmark. In the period May to September, freshly caught crabs were used. Prior to experiments the crabs were acclimated to experimental salinities and temperatures for 1 wk. Experimental salinities were made up by diluting Great Belt seawater (420 to 735 mOsm) with double distilled water. Little Belt and Great Belt seawater contains approximately 25 ng Cd l^{-1} (A. Jensen, pers. comm. 1985). The crabs were not fed during acclimation and experimental periods.

In 6 experimental series, groups of 4 to 6 crabs were exposed to cadmium (added as $CdCl_2 \cdot H_2O$) in 10 l polystyrene aquaria containing aerated seawater. Hemolymph parameters were followed in the groups of crabs exposed to lethal cadmium concentrations as long as 3 crabs were still alive. In each experimental series 5 to 12 crabs (4 to 6 per aquarium) in uncontaminated seawater served as running control groups. Table 1 summarizes experimental conditions in the different experiments carried out.

During the exposure periods, 0.2 ml hemolymph samples were drawn through the arthrodial membrane of the 4th periopod with hypodermic syringes. To prevent coagulation the hemolymph samples were immediately transferred to 0 °C and maintained at this temperature until osmolality measurements and dilutions for cation determinations had taken place. The remaining parts of the hemolymph samples were stored at -18 °C for subsequent chloride determinations.

Osmolality was measured with a Knauer semimicro osmometer and sodium, potassium, calcium, magnesium, and cadmium were determined by atomic absorption spectroscopy (Beckmann 1248 & Perkin-Elmer 2380). To eliminate interaction between the determination of individual ions, mixed standards with ion concentrations similar to crab hemolymph were employed. La_2O_3 was added to prevent phosphate interactions. Chloride determinations were performed with a Radiometer CMT 10 chloride titrator.

To investigate how cadmium was bound in the hemolymph, maximum obtainable hemolymph samples were drawn from the last surviving crab in the groups exposed to 6, 8, and 10 mg Cd l^{-1} in Experiment 6. At Day 46 in the exposure, hemolymph samples were likewise drawn from the surviving crabs exposed

Table 1. *Carcinus maenas*. Summary of experimental conditions in the various experiments

Experiment	Date of initiation	Exposure conc. (mg Cd l^{-1})	Osmolality (mOsm)	Temperature (°C)	n Exposed group	n Control group	Osm	Na	Cl	K	Ca	Mg	Cd	Median survival time (d)
		1	400±10		5 → 4	5 → 4	+	+	+	+	+	+		> 22[a]
1	21 Mar	1	550±20	18±1	5 → 5	5 → 5	+	+	+	+	+	+		> 22[a]
		1	700±20		5 → 5	5 → 5	+	+	+	+	+	+		> 22[a]
2	1 Jun	1	400±10	15.5±0.5	5 → 4	8 → 7	+	+	+	+	+	+	+	> 48[a]
		10			5		+	+	+	+	+	+	+	11
3	15 Feb	10	400±10	15.5±0.5	5	6 → 6	+	+	+	+	+	+	+	9
4	15 May	10	400±10	15.5±0.5	5	10 → 10	+	+	+	+	+	+	+	9
5	22 Aug	10	400±10	15.5±0.5	6	12 → 12	+	+	+	+	+	+	+	7
		10			6						+	+	+	16
		8			6						+	+	+	23
6	27 Aug	6	400±10	15.5±0.5	6	12 → 11					+	+	+	29
		4			6 → 2						+	+	+	46
		2			6 → 5						+	+	+	> 46[a]
		1			6 → 6						+	+	+	> 46[a]

[a] Period over which hemolymph parameters were followed

to 1, 2 and 4 mg Cd l⁻¹. One ml hemolymph samples (pooled or from single crabs) were run through a 60 cm × 0.9 cm Sephadex G-75 column. Twenty mM Tris (pH = 8.1) was used as elution buffer and absorbance at 280 nm and cadmium content were determined in each fraction collected.

For an *in vitro* investigation of cadmium binding in the hemolymph, a pooled sample from 15 crabs was used. The protein concentration in the hemolymph was 53 g l⁻¹ (Biuret method; Bergmeyer 1974). Concentrations of 0.5, 1, 2, 4, 8, 16, 32, 64, 128, and 256 μg Cd ml⁻¹ were added to 0.5 ml hemolymph samples, which were dialysed against 5.0 ml crab ringer (360 mM NaCl, 8 mM KCl, 22 mM $MgSO_4$, 4 mM $NaHCO_3$, 10 mM $CaCl_2$, pH = 8.0) at 4 °C. After 48 h the cadmium concentrations outside the dialysis bags were determined.

The statistical significance of differences between exposed groups and control groups were calculated using 2-tailed Student t-tests.

RESULTS

Crabs exposed to 1 and 2 mg Cd l⁻¹ did not show greater mortality than control groups (Table 1). Median survival times for crabs exposed to 4 to 10 mg Cd l⁻¹ are shown in Table 1

Osmolality and ion concentrations in the crabs at Day 0 are shown in Table 2. Osmolality and Na⁺ and Cl⁻ concentrations in crabs acclimated to 400 mOsm were lower in crabs kept at the field station during autumn and winter than in freshly caught crabs. Osmolality and Na⁺ and Cl⁻ concentrations in the control crabs decreased slightly during experimental periods, while K⁺, Ca⁺⁺, and Mg⁺⁺ levels showed no consistent changes.

Experiment 1

Crabs exposed to 1 mg Cd l⁻¹ at 400 to 700 mOsm (14 to 24 ‰) for 22 d showed no changes in hemolymph osmolality and Na⁺, Cl⁻, K⁺, Ca⁺⁺, and Mg⁺⁺ levels relative to control groups (data not shown).

Experiments 2 to 5

Crabs exposed to 1 mg Cd l⁻¹ at 400 mOsm for 48 d (Exp. 2) showed no changes in hemolymph osmolality and Na⁺, Cl⁻, K⁺, Ca⁺⁺, and Mg⁺⁺ concentrations relative to the control group (data not shown). The effect of 10 mg Cd l⁻¹ on these 6 parameters were not consistent in the 6 experiments (Fig. 1). In May and August exposure to 10 mg Cd l⁻¹ produced significant increases in hemolymph Na⁺ levels, and the same trend was seen in June. Increases were most pronounced after 2 to 4 d, after which a decline towards control levels was observed. Osmolality and Cl⁻ levels showed significant increases after 4 d exposure in the August experiment only. In the May experiment osmolality tended to increase. In the June experiment osmolality and Cl⁻ and K⁺ concentrations tended to decrease after 8 d exposure. K⁺ showed a significant increase after 4 d exposure in May and magnesium levels either showed an initial decrease (Feb and Jun) or they remained unaffected. Calcium levels rose to 160 to 180 % of controls after 7 to 8 d exposure to 10 mg Cd l⁻¹ in May, June, and August, while 5 d exposure did not affect calcium levels in February. The effect of cadmium on calcium levels appeared faster in August than in May and June.

In May, June, and August crabs exposed to 10 mg Cd l⁻¹ reached cadmium levels in the hemolymph of 20

Table 2. *Carcinus maenas*. Hemolymph osmolality and inorganic ion concentrations (mean ± SD) in crabs at Day 0 in each experiment

Experiment	Date	Osmolality (mOsm)	Na⁺ (mM)	Cl⁻ (mM)	K⁺ (mM)	Ca⁺⁺ (mM)	Mg⁺⁺ (mM)
1	21 Mar	649 ± 18	301 ± 24	326 × 21	8.1 ± 0.5	7.8 ± 0.9	10.0 ± 1.5
		726 ± 5[a]	351 ± 18	370 ± 11	8.7 ± 1.0	8.9 ± 1.1	12.0 ± 1.0
		795 ± 5[b]	376 ± 12	399 ± 14	8.7 ± 0.5	9.9 ± 0.4	15.0 ± 2.0
2	1 Jun	694 ± 40	361 ± 10	366 ± 30	8.2 ± 0.6	7.8 ± 0.6	11.7 ± 0.8
3	15 Feb	620 ± 35	310 ± 15	313 ± 27	8.2 ± 0.9	7.1 ± 0.4	9.2 ± 1.1
4	15 May	765 ± 23	384 ± 14	383 ± 22	8.3 ± 0.2	8.8 ± 1.0	11.3 ± 1.0
5	22 Aug	735 ± 36	349 ± 15	321 ± 32	8.5 ± 0.6	7.7 ± 0.8	10.4 ± 1.4
6	27 Aug	nd	nd	nd	nd	8.1 ± 1.1	11.1 ± 1.0

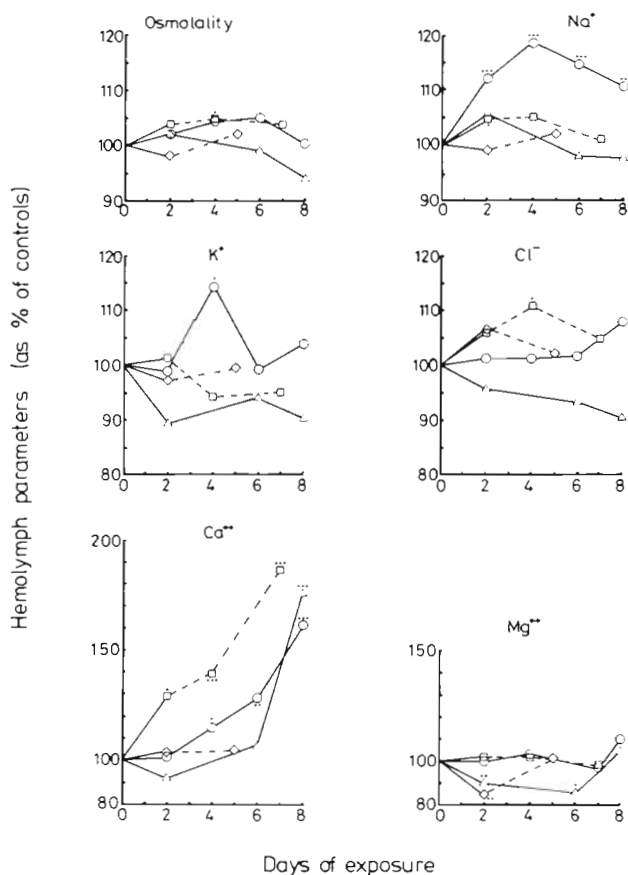[a,b] Acclimated to 550 and 700 mOsm respectively
nd Not determined

Fig. 1. *Carcinus maenas*. Hemolymph parameters in crabs exposed to 10 mg Cd l⁻¹. Mean values in all groups set to 100 % at Day 0. Parameters in exposed groups are expressed relative to values in control group during experiments. (△) 1 Jun; (□) 22 Aug; (◇) 15 Feb; (○) 15 May. Broken lines indicate death of 1 or several exposed crabs between sampling times. Numbers of specimens given in Table 1. (•), (••), and (•••) indicate differences from the control group at 5 %, 1 %, and 0.1 % level, respectively

to 32 μg Cd ml⁻¹ in 7 to 8 d, while uptake rates in February were lower (Fig. 2b). Crabs exposed to 1 mg Cd l⁻¹ (Jun) reached a hemolymph cadmium level of approximately 0.8 μg Cd ml⁻¹ within 1 wk and no further increase appeared during 48 d exposure (Fig. 2a).

Experiment 6

Cadmium levels in the hemolymph of crabs exposed to 1, 2, 4, 6, 8, and 10 mg Cd l⁻¹ are shown in Fig. 3a. Crabs exposed to 1 and 2 mg Cd l⁻¹ approached the ambient cadmium concentration in the first week and during 44 d exposure the cadmium concentrations in the hemolymph remained at or slightly below ambient concentrations. Crabs exposed to 4 mg Cd l⁻¹ reached levels of 8 to 10 μg Cd ml⁻¹ hemolymph after about
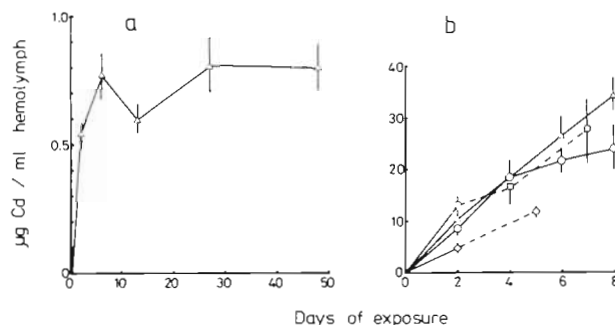

Fig. 2. *Carcinus maenas*. Hemolymph cadmium concentrations in crabs exposed to (a) 1 and (b) 10 mg Cd l⁻¹. Mean ± SEM. For explanation of symbols, see legend to Fig. 1

2 wk, and no further increase was seen. Cadmium concentrations in the hemolymph of crabs exposed to 6, 8, and 10 mg Cd l⁻¹ kept increasing until 22 to 36 μg Cd ml⁻¹ where death occured.

Calcium levels in crabs exposed to cadmium concentrations ≥4 mg Cd l⁻¹ increased immediately, while calcium levels in crabs exposed to 1 mg Cd l⁻¹ remained unaffected (Fig. 3b). Two mg Cd l⁻¹ seemed to cause an increase in calcium levels after prolonged exposure (Fig. 3b).

Magnesium levels in the hemolymph were not affected by exposure to 1, 6, 8, and 10 mg Cd l⁻¹, while 2 and 4 mg Cd l⁻¹ caused significant reductions in magnesium levels (Fig. 3c). After 44 d exposure the magnesium concentration in the group exposed to 4 mg Cd l⁻¹ was only 44 % of that in the control group.

Fig. 4 shows Sepadex G-75 elution profiles for hemolymph from crabs exposed to 1, 4, and 10 mg Cd l⁻¹. Recovery of hemolymph cadmium after gel filtration varied between 88 and 102 %. Cadmium was bound in the high molecular weight fraction and little cadmium enrichment was observed in the low molecular weight fraction.

In vitro experiment

When 0.5, 1, 2, 4, and 8 μg Cd was added per ml hemolymph *in vitro*, the concentrations of dialysable cadmium were below the detection limit (≈50 ng free Cd [ml hemolymph]⁻¹). The concentrations of dialysable cadmium in the hemolymph after addition of 16, 32, 64, 128, and 256 μg Cd ml⁻¹ are shown in Fig. 5a. After addition of 16 μg Cd ml⁻¹, 0.19 μg Cd ml⁻¹ is dialysable and the increase in dialysable cadmium for higher amounts of cadmium added show complex kinetics (in between linear and exponential increase). The number of cadmium ions bound to each hemocyanin molecule increases rapidly for low concentrations of free Cd^{++} (Fig. 5b). The number of cadmium ions
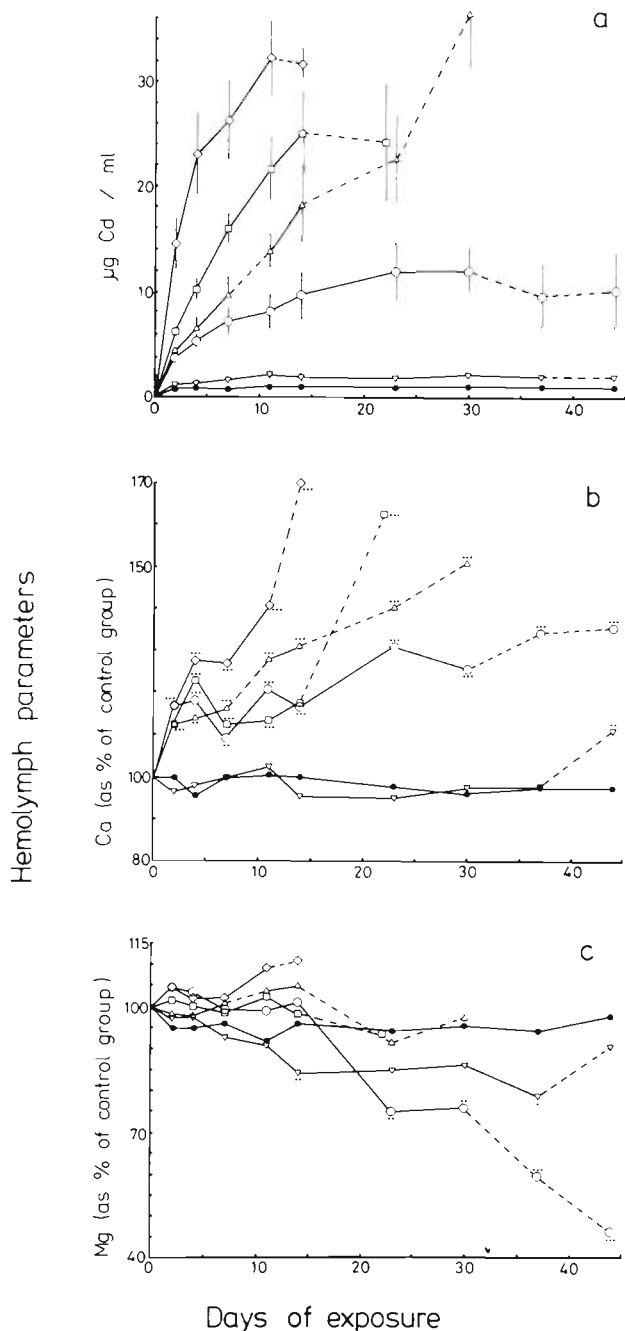
Fig. 3. *Carcinus maenas*. Hemolymph (a) cadmium, (b) calcium, and (c) magnesium concentrations in crabs exposed to (●) 1, (▽) 2, (○) 4, (△) 6, (□) 8, and (◇) 10 mg Cd l^{-1}. Broken lines indicate death of 1 or several crabs between sampling times. Symbols in (a) represent mean ± SEM. (b) and (c) expressed as explained in legend to Fig. 1

Fig. 4. *Carcinus maenas*. Sephadex G-75 elution profiles for hemolymph from crabs exposed to: (a) 1 mg Cd l^{-1} for 46 d (1.0 ml pooled from 6 crabs); (b) 4 mg Cd l^{-1} for 46 d (1.0 ml pooled from 2 crabs), and (c) 10 mg Cd l^{-1} for 17 d (1.0 ml from 1 crab). Dotted lines: A_{280}; solid lines: Cd. Detection limit for cadmium: 5 ng Cd ml^{-1}. Arrows indicate elution positions for chymotrypsinogen A (MW = 25 000) and cytochrome c (MW = 12 500)

Fig. 5. *Carcinus maenas*. (a) Partition of cadmium between dialysable and non-dialysable fraction after *in vitro* addition of cadmium to crab hemolymph. Mean ± SEM for 2 determinations. (b) Number of cadmium ions bound to each hemocyanin molecule as a function of the concentration of free cadmium ions. Calculated from means in Fig. 5a

bound to each hemocyanin molecule seems to be determined by saturation kinetics, although saturation of hemocyanin with cadmium was not achieved. The hemocyanin concentration was calculated from the total protein content in the hemolymph (approximately 95 % hemocyanin, MW = 75 000; Zatta 1984).
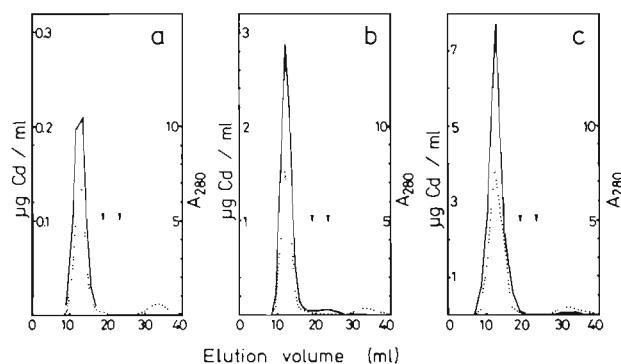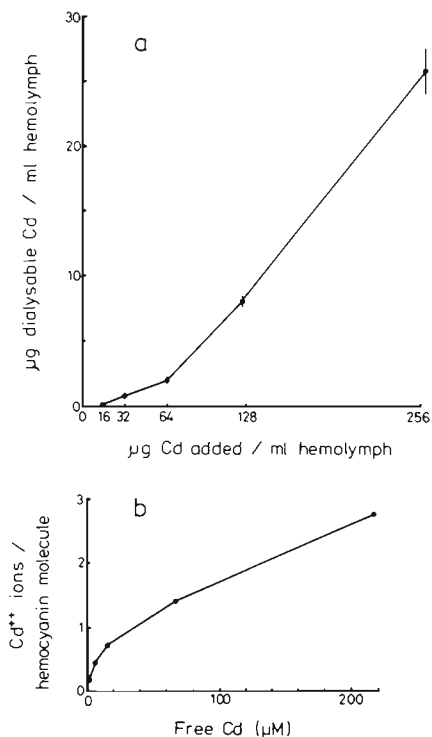
DISCUSSION

In the present study 1 mg Cd l^{-1} (at 400 mOsm and 18 °C) was not lethal in 22 d. Wright (1977a) likewise found that 2.4 mg Cd l^{-1} (at 500 mOsm and 10 °C) was not lethal in 68 d, while Thurberg et al. (1973) found

that 1 mg Cd l⁻¹ (at 450 mOsm and 19 to 22 °C) was lethal in 48 h. Furthermore, Thurberg et al. (1973) observed increases (maximally 7 %) in hemolymph osmolalities in crabs exposed to 0.5, 1, 2, 4, and 8 mg Cd l⁻¹ at 450, 600, 700, 800, and 900 mOsm for 48 h, while in the present study exposure to 1 mg Cd l⁻¹ caused no effect on hemolymph osmolalities at 18 °C and 400, 550 and 700 mOsm. However, the crabs of Thurberg et al. (1973) had been acclimated to 700 mOsm, and then simultaneously exposed to changed salinity and cadmium. Crabs not in osmotic equilibrium may be more sensitive to cadmium both with respect to mortality and effects on osmoregulation than crabs in osmotic equilibrium. Also, the variation in temperature between the 2 studies may explain the different results.

The small increase in osmolality in crabs exposed to 10 mg Cd l⁻¹ is in accordance with the results of Thurberg et al. (1973). Quantitatively, Na$^+$ and Cl⁻ are the most important inorganic ions in crab hemolymph and therefore changes in osmolality would be expected to be caused by changes in Na$^+$ and/or Cl⁻ concentrations. This is observed in most experiments (Fig. 1), but it is noteworthy that the pronounced increase in Na$^+$ concentrations in the May experiment is followed only by slight (and not significant) increase in osmolalities. The variation between individual experiments of the effects of 10 mg Cd l⁻¹ on osmolality and Na$^+$, Cl⁻, and K$^+$ concentrations could not clearly be attributed to dependence on time of year and thereby stage in the moult cycle.

The effects of prolonged cadmium exposure on magnesium regulation show a complex concentration dependence, since only 2 and 4 mg Cd l⁻¹, but not 1, 6, 8, and 10 mg Cd l⁻¹, reduce hemolymph magnesium levels (Fig. 3c). Prolonged exposure to copper concentrations between 0.25 and 1.0 mg Cu l⁻¹ cause similar reductions in magnesium concentrations in *Carcinus maenas* (Bjerregaard & Vislie 1985a). *C. maenas* normally maintains low hemolymph magnesium concentrations relative to ambient seawater by excreting magnesium via the antennal gland-bladder complex (Lockwood & Riegel 1969, Zanders 1980b). Cadmium may reduce hemolymph magnesium levels by: (1) altering gill permeability for magnesium, (2) augmenting urine production rates, or (3) stimulating magnesium secretion in the bladder, but the present data do not indicate which mechanism is the most probable one.

Exposure to cadmium concentrations ≥4 mg Cd l⁻¹ augmented hemolymph calcium levels. Wright (1977c) found an augmenting effect on hemolymph calcium levels of 2.4 mg Cd l⁻¹ after 6 d in postmoult crabs, but not in intermoult crabs.

Cadmium could augment hemolymph calcium levels by: (1) stimulating calcium transport over the gills from seawater to hemolymph, (2) inducing release of calcium from calcium-containing organs (exoskeleton and hepatopancreas) to the hemolymph, (3) interfering with the hormonal regulation of moulting and thereby calcium metabolism (Robertson 1937, 1960), or (4) diminishing urine production rates.

In the freshwater amphipod *Gammarus pulex*, 0.6 mg Cd l⁻¹ reduce calcium uptake (Wright 1980) and the activity of the Ca^{2+} ATPase extracted from gills of roach *Rutilus rutilus* are inhibited *in vitro* by heavy metals (Shephard & Simkiss 1978). It is thus unlikely that cadmium should stimulate calcium uptake via the gills in *Carinus maenas*.

As for the second possibility, Roer (1980) has shown that active transport of calcium across the hypodermis takes place by means of a Ca-ATPase and a Na/Ca exchange mechanism. If cadmium inhibits active transport of calcium from hemolymph to exoskeleton, hemolymph calcium levels would increase due to passive influx of calcium from the exoskeleton (Roer 1980). Cadmium could then reduce active calcium transport either directly by inhibiting the Ca-ATPase or indirectly by inhibiting the ouäbäin sensitive Na$^+$ transport and thereby the Na/Ca exhange pump. Transport of calcium from the hemolymph to the exoskeleton is approximately 8 times higher in postmoult crabs than in intermoult crabs (Roer 1980). This may explain why Wright (1977c) found an effect of cadmium on calcium regulation in postmoult crabs, but not in intermoult crabs. Adult male crabs in Danish waters moult in June to July (Rasmussen 1973) and in the present study effects of 10 mg Cd l⁻¹ on hemolymph calcium levels tend to appear faster in the August experiment (late postmoult) than in May and June (premoult).

The effect of cadmium on hemolymph calcium levels resembles that caused by injecting moult-promoting hormone from the Y-organ (Carlisle 1957). In the intermoult stage secretion of the moult-promoting hormone is inhibited by the moult-inhibiting hormone – a polypetide – produced in the X-organ-sinus gland complex (Carlisle 1957). If cadmium affects the production or function of the moult-inhibiting hormone, the observed changes in calcium levels would be expected in association with the partial resorption of the exoskeleton initiating the moult processes (Robertson 1937, Roer 1980). However, this hypothesis cannot explain why Wright (1977c) found an effect of cadmium on calcium levels in postmoult but not in intermoult crabs.

Toxic concentrations of copper and mercury also augment hemolymph calcium levels in *Carcinus maenas* (Bjerregaard & Vislie 1985a, b) and the interactions of heavy metals with the complex calcium metabolism of *C. maenas* bear further investigations.

It is in accordance with the observations of Wright (1977a, b) and Wright & Brewer (1979) that *Carcinus maenas* exposed to ≤ 2 mg Cd l^{-1} maintain hemolymph cadmium concentrations at or slightly below ambient concentrations. Crabs exposed to ≥ 4 mg Cd l^{-1} concentrate cadmium in the hemolymph to concentrations that are higher than ambient cadmium concentrations and *C. maenas* does not seem to be able to survive with hemolymph cadmium concentrations that are higher than 35 to 40 µg Cd ml^{-1}. It is noteworthy that at these hemolymph cadmium concentrations, substantial amounts of free cadmium start to appear in the hemolymph. Likewise, the effect of cadmium on hemolymph calcium levels also seems to be related to the hemolymph cadmium concentration (Fig. 3a, b).

Jennings et al. (1979) and Rainbow & Scott (1979) demonstrated 2 proteins (MW = 12 500 and 27 000) in *Carcinus maenas* with a high affinity for cadmium. Jennings et al. (1979) suggest that cadmium entering the hemolymph via the gills is transported to other organs – mainly hepatopancreas – bound to low molecular weight proteins. The present study, however, shows that the major part of the cadmium present in the hemolymph is bound in the high molecular weight fraction – probably to hemocyanin, which constitutes more than 95 % of the protein in crab hemolymph (Uglow 1969, Zatta 1984). Likewise, Zatta (1984) – based on the metabolism of zinc in *C. maenas* – suggests that hemocyanin can act as a metal carrier in the hemolymph. Wright & Brewer's (1979) results indicate that the turnover time for hemolymph cadmium is relatively short (approximately 50 % of the cadmium exchanged in 40 h) and Zatta (1984) found similar values for zinc. Thus, with the very limited amount of cadmium bound in the low molecular weight fraction it does not seem likely that this fraction could act as the most important vector for cadmium transport in the hemolymph, although the turnover time for cadmium in this fraction may be very fast. However, the mechanism for regulation and transport of cadmium in the hemolymph of *C. maenas* needs further clarifying.

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