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Effect of the Amino Acid Histidine on the Uptake of Cadmium from the Digestive System of the Blue Crab, *Callinectes sapidus*

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Heavy metals, such as cadmium, are important pollutants in many coastal waters (BRULAND et al. 1974; WILLIAMS et al. 1978). At sublethal concentrations, cadmium has been shown to affect a number of metabolic processes in marine invertebrates. For example, cadmium altered the swimming rates, delayed the growth, and increased the mortality of the larvae of the crab Eurypanopeus depressus (MIRKES et al. 1978); retarded limb regeneration and ecdysis in the crab Uca pugilator (WEISS 1978); and depressed oxygen consumption of the gill tissues of the crabs Carcinus maenas and Cancer irroratus (THURBERG et al. 1973). The toxicity of cadmium in marine invertebrates is influenced by changes in many chemical and physical parameters such as salinity and temperature (O'HARA 1973; HUTCHESON 1974; ROSENBERG & COSTLOW 1976) and the availability of chelators in seawater or sediment (PHELPS 1979).

The biological processes determining the rates and sites of incorporation, transportation, detoxification, and depuration in animals are also parameters affecting a metal's toxicity. For example, the processes involved in digestion and absorption of food may be crucial in those animals where the main entry route for the metal is via the alimentary canal (e.g. PATRICK & LOUTIT 1978; JENNINGS & RAINBOW 1979 a,b). An important product of digestion that has been shown to affect the absorption of ingested heavy metals, including cadmium, is free amino acids. In rat intestine, the presence of certain amino acids, such as histidine, lysine and cysteine, enhanced the transport of iron and other metals across the mucosal epithelium (VAN CAMPEN 1973; SMITH et al. 1978). FORTH et al. (1974) observed that histidine enhanced metal absorption by the mucosal epithelium of rat intestine at low histidine:metal concentration ratios, but inhibited metal uptake at higher concentration ratios. The proposed mechanism of the amino acid-metal interaction is that the amino acid complexes with the metal cations and prevents the formation of metal hydroxides and phosphates which are poorly absorbed by the mucosal epithelium. Current research suggests that interactions between metals and amino acids occur in the tissues of marine invertebrates as well. The potential importance of chelation and detoxification of metals by amino acids and other organic molecules has been suggested by a number of studies in a variety of organisms. For example, the presence of dissolved cadmium reduced the uptake of glycine across the epidermis of two species of polychaetes (SIEBERS & EHLERS 1978) and caused a decrease in the total intracellular free amino acid pool in Mytilus edulis (BRIGGS 1979). In view of the increasing emphasis being given to chelation phenomena in biological systems and the importance of the alimentary canal in metal

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incorporation, it is important to determine whether amino acids enhance metal absorption across the gut wall in invertebrates. This phenomenon has been examined in the blue crab, Callinectes sapidus, in this study.

MATERIALS AND METHODS

Individual Callinectes sapidus were obtained from the salt marsh pools and tidal creeks along East Beach and Interstate 45 near Galveston, Texas and from Bastrop Bayou near Freeport, Texas. A baited crab cage proved the best method of capture. The collected crabs were placed individually in buckets of seawater and transported to the laboratory. There, the animals were maintained in 15°/₀₀ seawater and fed frozen shrimp twice a week. Crabs having a carapace width of 10.2±2.4 cm and carapace length of 5.1±1.3 cm were used for these experiments. In order to facilitate handling, the chelipeds were inactivated by cutting the muscle at the suture line of the propodite segment.

To optimally trace the absorption of cadmium as a function of histidine concentration, the hepatopancreas, being the site of absorption, should be directly monitored. Due to the complex system of tubules in the hepatopancreas, however, it was not practical to do so on living crabs. An indirect method was devised where known amounts of histidine or cadmium were injected into the cardiac stomach and their subsequent appearance in the hemolymph monitored. The crabs were prepared prior to the experiments by gently boring a hole into the dorsal carapace directly above the position of the cardiac-stomach. A 50 µl solution of cadmium and histidine was injected into the animal by putting the syringe tip through the hole and puncturing the cardiac-stomach.

Solutions with the following µM histidine:µM cadmium ratios were used: 20:0.05, 10:0.1, 1:0.1, 0.1:0.1, and 0:0.1, hereafter termed treatments 400:1, 100:1, 10:1, 1:1, and 0:1, respectively. Depending upon the particular experiment, either ^{14}C-histidine or ^{109}Cd was used as a tracer. ^{109}Cd was used in treatments 400:1, 100:1, 10:1, 1:1, and 0:1. In order to compare the absorption of cadmium with histidine, ^{14}C-histidine was substituted for ^{109}Cd in repetitions of treatments 100:1 and 1:1. At 5,30,60,90,120,150,180,210, and 240 minute intervals after injection an aliquot of hemolymph was removed from the animal using a 1 ml tuberculin syringe. The site of withdrawal was the base of the fifth periopod of the crab (CANTELMO et al. 1975). The sample was placed in a 0.5 ml polypropylene tube with distilled water. The tube was put inside a scintillation vial and counted on a Beckman liquid scintillation counter using Tritisol (FRICKE 1975) as the scintillation cocktail. When ^{14}C-histidine was used, the hemolymph aliquot was mixed with 1 ml of distilled water and put directly into the Tritisol.

RESULTS

The mean value and standard error for each time interval in all treatments using both ^{109}Cd and ^{14}C-histidine are listed in Table 1. The mean values for treatments in which ^{109}Cd was used are plotted in

Figure 1. One feature common to all five treatments is that cadmium was absorbed within the first 5 minutes. In treatment 1:1 and in treatment 10:1, the concentration of cadmium was higher in the first 5 minutes than in the remaining treatments. Treatments 400:1, 100:1, and 0:1 showed virtually no change from the initial 5 minute amount over time. In treatment 0:1 and 100:1, cadmium falls to 50% of the 5 minute value at 210 minutes and 180 minutes, respectively. In comparison, the cadmium concentration in treatment 1:1 dropped to 50% of the 5 minute value before 30 minutes, and treatment 10:1 by 120 minutes.

TABLE I

Callinectes sapidus. Mean concentration of cadmium and histidine in the hemolymph of all treatments. Each quantity is the mean concentration ± the standard error and expressed as nmoles/ml hemolymph.

Time (minutes)	Cadmium n=6, 0:1	Cadmium n=8, 1:1	Cadmium n=6, 10:1	Cadmium n=6, 100:1	Cadmium n=6, 400:1	Histidine n=5, 1:1	Histidine n=4, 100:1
5	4.0±0.4	14.2±2.0	5.4±1.8	3.0±1.1	2.0±0.4	2.3±0.5	301.3±57.7
30	3.6±0.4	7.3±1.1	4.4±0.6	2.5±0.7	2.8±0.5	1.8±0.4	236.9±54.0
60	3.4±0.4	5.5±0.7	4.0±0.7	2.4±0.4	2.5±0.4	1.7±0.4	207.8±52.2
90	3.9±0.8	5.0±0.8	3.0±0.3	2.0±0.3	2.2±0.4	1.8±0.3	185.9±38.1
120	2.1±0.3	4.5±0.6	2.6±0.4	2.2±0.3	1.5±0.4	1.6±0.5	150.8±25.6
150	2.4±0.5	4.2±0.7	2.6±0.3	2.3±0.5	2.1±0.5	1.3±0.2	176.8±29.3
180	2.0±0.2	3.4±0.6	2.2±0.3	1.4±0.8	2.0±0.3	1.3±0.1	150.0±22.1
210	2.0±0.2	2.8±0.5	1.6±0.5	1.8±0.5	1.2±0.1	1.4±0.2	147.2±27.6
240	2.3±0.2	2.4±0.4	1.1±0.5	1.2±0.1	1.4±0.4	1.2±0.6	130.6±36.9

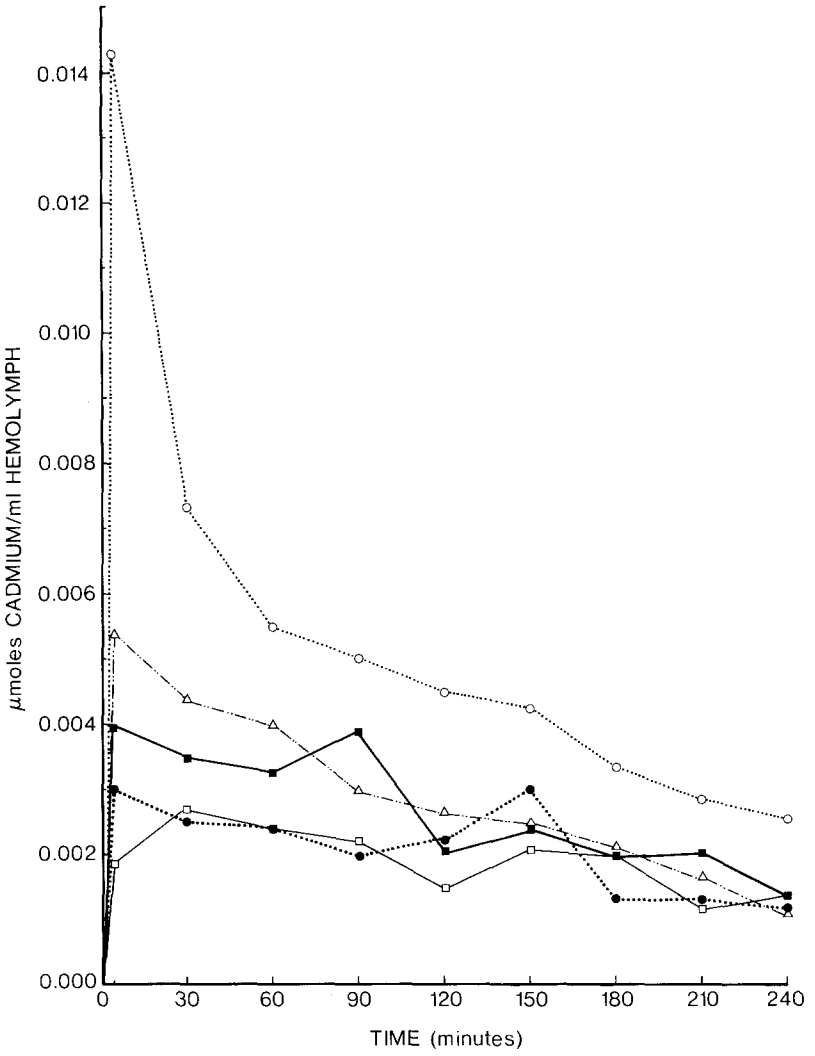

FIGURE 1. The mean concentration of cadmium (μmoles Cd/ml hemolymph) in the hemolymph of the blue crab for all Cd:histidine ratios:
o, 1:1 ■, 0:1 ●, 100:1 □, 400:1 Δ, 1:10

The greatest amount of cadmium uptake into the hemolymph occurred in treatment 1:1. A maximum mean value in the first 5 minutes of 14.2 ηmoles/ml hemolymph was recorded. In other treatments, the maximum cadmium levels in the hemolymph were considerably lower; treatment 10:1 at 5.4 ηmoles/ml, and treatments 0:1, 100:1, and 400:1 clumped between 2.0 ηmoles/ml and 4.0 ηmoles/ml.

A large difference in the uptake of ^{14}C-histidine was observed in treatments 100:1 and 1:1. The mean values for each time interval and the standard error are given in Table 1 and plotted in Figure 2. The maximum level of histidine in the hemolymph occurred in the first 5 minutes for both treatments. The mean value found for treatment 1:1 (2.3 ηmoles/ml hemolymph) was roughly 100 times less than the mean value for treatment 100:1 (301.3 ηmoles/ml). In treatment 100:1, the histidine level in the hemolymph dipped to 50% of the 5 minute value after 120 minutes, whereas in treatment 1:1, histidine in the blood stayed at concentrations greater than 50% for the entire experiment.

At 5 minutes, the amount of cadmium in the hemolymph for treatment 1:1 was significantly higher than that measured in treatments 0:1, 10:1, 100:1 and 400:1 (T-test with α=.05). The hemolymph cadmium levels were statistically the same for treatments 0:1, 10:1, 100:1 and 400:1 at all time intervals, although cadmium hemolymph concentrations for treatment 10:1 appeared higher at 5 minutes than in the other three treatments (see Figure 1). This suggests a gradual decrease in cadmium uptake at 5 minutes occurred with increasing histidine concentration. Interestingly, the same low cadmium transport into the hemolymph occurred at both the high histidine:cadmium ratios (400:1, 100:1) and when cadmium alone was injected into the cardiac-stomach.

A comparison between histidine and cadmium levels at the start and the conclusion of treatment 100:1 and 1:1 shows that the processes governing uptake of the metal and the amino acid were different (Table 1). As expected, the amount of histidine entering the hemolymph appears to be concentration-dependent. At 5 minutes, 2.3 ηmoles/ml was measured at the 1:1 concentration ratio, and 301.3 at the 100:1 ratio. In contrast, the amount of cadmium transported into the hemolymph under the same conditions was sharply different. There was almost 5 times less cadmium per ml hemolymph found for treatment 100:1 than for treatment 1:1, even though the same amount of cadmium had been injected into the crabs for both these treatments. The results of treatment 0:1 showed that the amount of the injected cadmium transported into the hemolymph was similar to treatment 100:1, rather than 1:1.

DISCUSSION

The effect of histidine on the uptake of cadmium into the hemolymph of Callinectes sapidus is dependent on the processes governing digestion and absorption which, in crustaceans, occur primarily in the digestive gland (GIBSON & BARKER 1979). The test solution of concentrated cadmium and histidine was injected as a single dose directly into the cardiac-stomach. The crabs, in all the treatments, responded in the same way with a pulse of cadmium and histidine appearing rapidly in the hemolymph. The results show the net effect of histidine on cadmium absorption and transport from the alimentary

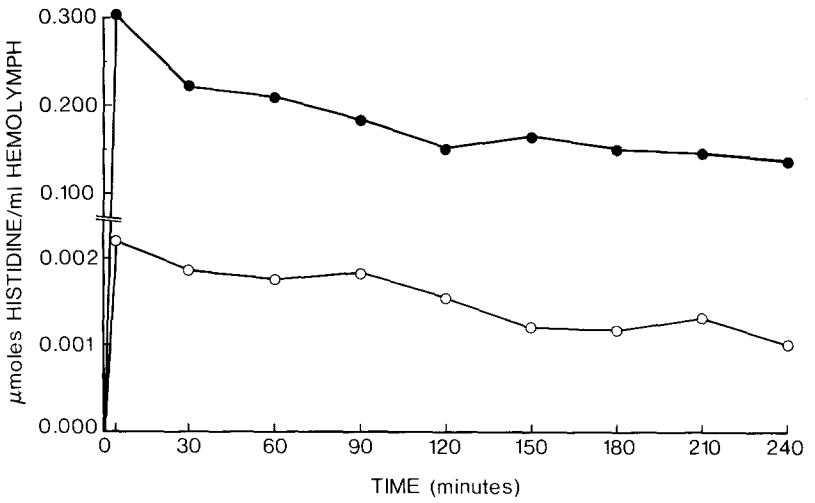

FIGURE 2. The mean concentration of histidine in the hemolymph of crabs used in treatments 100:1 (•) and 1:1 (o).

canal to the hemolymph. The variation in cadmium influx rates with varying histidine concentrations parallels what has been previously elucidated in vertebrates (FORTH et al. 1974). In both cases using similar methodologies, low histidine:metal ratios resulted in increased metal transport over that observed without amino acid. In both cases, higher histidine:metal ratios yielded lower metal transfer rates. Although various hypotheses have been proposed, the evidence in vertebrates suggests that metal chelation by amino acids is responsible for the observed results.

The pheonomenon of metal chelation by amino acids is well-known (GREENSTEIN & WINITZ 1961). VAN CAMPEN (1973) showed in rat intestine that histidine, cysteine, and lysine kept iron in solution by forming ligands with the metal, for example. It is probable, therefore, that the formation of histidine-cadmium chelates explains the results observed here. Two alternatives exist. Chelation may affect the amount of cadmium in solution and available for cellular absorption; or chelation may allow cadmium to be co-transported with histidine during cellular absorption processes.

In the first case, histidine could either maintain cadmium in solution and increase cadmium absorption by preventing the in vivo formation of relatively insoluble inorganic compounds of cadmium; or histidine might prevent absorption by removing free cadmium from solution. If histidine maintains cadmium, which might otherwise be bound in a less available form, in solution and available for absorption, cadmium uptake rates should increase with elevated histidine: cadmium ratios. This was not observed. On the other hand, if the histidine-cadmium complex effectively removes free cadmium from solution and prevents its absorption, declining cadmium uptake rates

with increasing histidine:cadmium ratios would be expected. This, too, was not observed, since uptake rates for treatment 0:1 (no histidine) were no higher than for treatments 100:1 and 400:1. The observed increase in cadmium uptake only at low histidine-cadmium ratios cannot be explained by a mechanism in which histidine simply alters the amount of cadmium available for absorption.

The second alternative is that the histidine:cadmium complex competes with unchelated histidine. In this case, in the absence of histidine, cadmium transport would occur relatively slowly. With low histidine:cadmium ratios, transport should increase as cadmium is co-transported with histidine. At high histidine:cadmium ratios, however, cadmium absorption decreases as free histidine competes with the histidine-cadmium complex for absorption. The resulting cadmium uptake rates should be low without histidine, high at low histidine:cadmium ratios and low again at high histidine:cadmium ratios. This is the observed result. In comparison to the effect histidine has on cadmium uptake, histidine transport is relatively unaffected by the presence of cadmium at the concentration ratios used. In treatment 1:1, when both histidine and cadmium were in equimolar concentrations, the amount of histidine in the hemolymph was approximately 1% of that found in treatment 100:1. This suggests that the complex is co-transported at roughly the same rates and in the same manner as unchelated histidine. Thus, the results suggest that histidine chelates with cadmium and that the amino acid:metal complex competes with unchelated histidine for absorption by the mucosal epithelium. Consequently, under advantageous low amino acid:metal ratios, absorption of metal can be greatly enhanced.

The digestive tract functions in the storage, metabolism, and execretion of heavy metals in invertebrates (e.g. JANSSEN & SCHOLZ 1979; GEORGE et al. 1980; GUARY & NEGREL 1980). The importance of the digestive tract and the processes governing digestion and absorption of nutrients in heavy metal uptake is becoming increasingly clear (e.g. VAN CAMPEN 1973; FORTH et al. 1974; GEORGE & COOMBS 1977; JENNINGS & RAINBOW 1979a,b; PHELPS 1979). The results of this study suggest that in order to understand the processes controlling heavy metal uptake in invertebrates, it will be necessary to investigate the role that digestion and absorption play in determining the transport rate of metals across the gut wall into the blood. For example, some amino acids increase metal absorption rates, whereas other compounds, such as phytate, decrease metal absorption rates. The results also suggest that experimental designs to investigate metal absorption must include an appreciation of the significant role that the feeding state of the animal (e.g. fed or starved) and the role chelators, particularly those produced by the organisms themselves during digestion, may play in the observed uptake rates of metal ions.

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