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# Effects of Cadmium on the Intracellular Pool of Free Amino Acids in *Mytilus edulis*

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Cadmium has been demonstrated to cause a wide variety of physiological and biochemical effects in aquatic organisms. Exposure to cadmium caused increased serum osmolality, significantly decreased serum calcium, and significantly increased serum magnesium in Pleuronectes flesus (LARSON et al. 1976). In Cancer irroratus, cadmium chloride exposure caused a significant increase in heart aminotransferase activity and elevated serum magnesium (GOULD et al. 1976). In a seven-day experiment with Mytilus edulis, 0.5 ppm cadmium caused a 50% reduction in the mean number of byssal threads produced (MARTIN et al. 1976).

Bivalve molluscs exposed to extended periods of reduced salinities volume regulate by the loss of intracellular solutes (PIERCE 1971). The solutes lost in this isosmotic intracellular regulation are primarily free amino acids (GILLES 1972). My study was undertaken to determine if the presence of cadmium would alter free amino acid content of Mytilus edulis.

## MATERIALS AND METHODS

The mussels were donated by Abandoned Farm, Inc. of Damariscotta, Maine, the first large-scale mussel culture operation in the United States. The Damariscotta River is an extremely clean estuary and the mean (n=12) background cadmium level in the mussels was 0.20 ± 0.02 (SE) ug/g wet weight. The mussels used were 48-52 mm in length. Prior to the experiment, the mussels were kept in 30°/oo salinity at 11°C for two weeks.

The three salinities used in the experiment, 30°/oo, 11°/oo, and 6°/oo were made by diluting Instant Ocean with deionized water. (The deionized water contained no detectable cadmium; however, 30°/oo Instant Ocean contained 50 ppb cadmium). At each salinity, tanks were made up containing 0, 0.1, 1.0, and 5.0 ppm additional cadmium; the chloride salt was used to make the test solutions. Each of the 12 tanks contained 20 l of saltwater. Twenty-five mussels were placed in each tank at the beginning of the experiment which was run at 11°C. Whenever possible, five mussels were removed after 20 hours, 40 hours, 208 hours, 376 hours, and 544 hours.

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Due to mortality in some aquaria, fewer than five animals remained for the 376 and/or 544 hour samples. In such cases, all the remaining animals were removed.

The posterior adductor muscle was removed intact and gently blotted dry. The free amino acids (FAA) were extracted in saturated picric acid and the acid solution slowly run through 1.0 x 4 cm columns of Dowex 50W-X8 ($H^+$) (KASSCHAU 1975). The FAA were eluted from the column with 2N $NH_4OH$. The FAA were derivatized (N-Trifluoro-acetyl n-butyl esters) and quantified by gas chromatography (ROACH and GEHRKE 1969a,b). Each sample was run in duplicate and the average of each amino acid was determined. The mean FAA concentrations were calculated for mussels from each tank at each sampling time.

The numerical data was analyzed by two way factorial analysis of variance. For any animal that died due to the experimental treatment, zero was entered into the analysis of variance calculation (SNEDECOR and COCHRAN 1969). This was not a consideration until the 376 hour sampling by which time seven animals had died. By the 544 hour sampling, 27 animals had died and this period was not tested by analysis of variance. At each salinity x cadmium x time combination, the Student's t-test was used to compare the mean values of alanine, glycine, and the total FAA osmolarity of the cadmium-exposed animals and the control animals.

The cadmium analysis was done by flameless atomic absorption. The cadmium concentration of every tank was checked once a week and there were no statistically significant differences from the original value within the experimental error of 4%. The mussel tissue was wet-ashed by placing it in a 5:1 nitric acid:perchloric acid solution and gently heating it until there was no foam or particulate matter present. When the solution was clear, it was heated until almost dry and then the volume was taken to 10 ml with distilled-deionized water.

## RESULTS AND DISCUSSION

Qualitative observations: The mussels in the 30°/oo 1.0 ppm and 5.0 ppm cadmium aquaria produced very few byssal threads. In contrast, the mussels in the 30°/oo salinity control and 0.1 ppm cadmium aquaria produced many byssal threads. These observations are in agreement with MARTIN et al. (1976) who found that 0.5 ppm cadmium caused a 50% reduction in the mean number of byssal threads produced. The mussels in the 11°/oo and 6°/oo salinity water did not produce byssal threads. It has been shown that Mytilus edulis acclimated to 32-34 °/oo do not produce byssal threads when transferred to 16°/oo (VAN WINKLE 1970) and so production of byssal threads would not be expected in greater dilutions of saltwater.

The test solutions at 30°/oo salinity containing 1.0 ppm and 5.0 ppm cadmium became yellow within 20 hours and foam appeared on the surface of the 5.0 ppm tank. These conditions lasted for the duration of the experiment. The 11°/oo and 6°/oo test solutions remained clear for the duration of the experiment. GEORGE and COOMBS (1977) found that 0.7 ppm cadmium stimulated marked mucus production which resulted in foam on the surface of the water. They considered this to be the first visible sign of cadmium toxicity.

Quantitative results: It is apparent from this study that after 208 hours exposure, cadmium has significantly affected alanine, glycine, and the total FAA osmolarity (Table 2). There are no statistically significant cadmium-salinity interaction effects at this time. The analysis of variance indicated that after 376 hours exposure, cadmium and cadmium-salinity interaction had significantly effected the concentration of virtually every FAA. Table 1 shows the magnitude of this effect. The Student's t-test (Table 3) indicated that in 30°/oo salinity, the presence of cadmium caused statistically significant reductions in FAA concentrations beginning after 208 hours exposure and lasting until the end of the experiment. Figure 1 shows the magnitude of these reductions after 376 and 544 hours exposure. In the 11°/oo salinity series, the cadmium-exposed animals had lower levels of FAA than the controls; these reductions were not consistently significant until 544 hours exposure. Figure 1 shows the magnitude of the reduction after 544 hours exposure. Within each salinity series, there were no consistent dose dependent effects.

BOYDEN (1977) has shown that the cadmium content in Mytilus edulis is directly related to body weight and concentration is independent of body size. He hypothesizes that this could be a result of cadmium "binding by specific chemical constituents whose concentrations are themselves directly related to body size". NOEL-LAMBOT (1976) has demonstrated in a laboratory study, that chronic low-level cadmium exposure can stimulate the synthesis of low molecular weight cadmium-binding proteins in Mytilus edulis. In mammalian systems, cadmium-binding proteins (metallothioneins) are thought to act as a detoxication system (WINGE and RAJAGOPALAN 1972).

The results of my study are consistent with the assumption that the saturation of the detoxication system in M. edulis occurs at about 1 ppm cadmium in 30°/oo water. This would explain the lack of consistent dose dependent effects of 0.1 and 1.0 ppm cadmium, the release of yellow pigment in the 1.0 and 5.0 ppm cadmium solutions, and the high mortality in the 5.0 ppm aquaria. GEORGE and COOMBS (1977) found that 0.7 ppm cadmium saturated the detoxication system of M. edulis and this coincided with the first visible signs of cadmium toxicity.

TABLE 1

Numerical results for alanine, glycine, and total FAA osmolarity after 376 hours exposure. Alanine and glycine together constituted greater than 50% of the total FAA osmolarity and thus were examined individually. The amino acids are mM/kg wet weight and the total FAA is mOsm/kg tissue water. The cases where mortality prevented taking a sample are indicated by "D". The cadmium levels indicated do not include the cadmium present in Instant Ocean.

| Salinity (°/oo) | | control | | | 0.1 ppm Cd | | | 1.0 ppm Cd | | | 5.0 ppm Cd | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 30 | | $\bar{x}$ | SD | n | $\bar{x}$ | SD | n | $\bar{x}$ | SD | n | $\bar{x}$ | SD | n |
| | Alanine | 37.80 | 6.73 | 5 | 25.64 | 5.59 | 4 | 19.20 | 3.42 | 4 | D | | |
| | Glycine | 127.53 | 34.10 | 5 | 85.25 | 14.37 | 5 | 36.74 | 10.16 | 5 | D | | |
| | Total FAA | 266.37 | | | 193.90 | | | 117.00 | | | D | | |
| 11 | | | | | | | | | | | | | |
| | Alanine | 17.49 | 6.25 | 5 | 11.01 | 6.98 | 5 | 15.25 | 0.88 | 4 | 18.29 | 12.03 | 5 |
| | Glycine | 32.57 | 23.45 | 5 | 28.44 | 10.47 | 5 | 22.56 | 11.68 | 5 | 35.83 | 8.10 | 5 |
| | Total FAA | 78.91 | | | 64.19 | | | 68.89 | | | 87.60 | | |
| 6 | | | | | | | | | | | | | |
| | Alanine | 10.75 | 1.03 | 3 | 17.34 | 1.81 | 4 | 19.84 | 3.80 | 5 | 16.59 | 4.87 | 5 |
| | Glycine | 39.37 | 12.21 | 3 | 42.57 | 14.18 | 5 | 35.42 | 9.49 | 5 | 41.63 | 10.61 | 5 |
| | Total FAA | 86.14 | | | 96.39 | | | 95.23 | | | 111.82 | | |

TABLE 2

Summary of the two way factorial analysis of variance after 208 and 376 hours of exposure to cadmium. The "*" indicates a significant effect due to a factor. The level of significance is 0.01.

| Time | Factor | FAA | Ala | Val | Gly | Ile | Leu | Pro | Thr | Phe | Asp | Glu | Lys |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 208 Hr. | Salinity | * | * | * | * | * | | * | | | | | |
| | Cadmium | * | * | | * | | | | * | | | | * |
| | Interaction | | | | | | | | | | | | |
| 376 Hr. | Salinity | * | * | * | * | * | * | | | | * | | |
| | Cadmium | * | * | * | * | * | * | * | | * | * | * | * |
| | Interaction | * | * | * | * | * | * | * | * | * | * | * | * |

TABLE 3

Student's t-test comparison of the control and experimental alanine, glycine, and total FAA osmolarity after 208, 376, and 544 hours of exposure to cadmium. An "*" indicates a significant difference from the control mean at a significance level of 0.05. The "+" indicates the $\bar{x}_{exp} > \bar{x}_{cont}$, a "-" indicates the $\bar{x}_{exp} < \bar{x}_{cont}$. The cases where mortality prevented taking a sample are indicated by "D"; the "CD" indicates the controls were dead and no comparison was possible. The cadmium concentrations indicated do not include the background cadmium present in Instant Ocean.

| Salinity (°/oo) | | 30 | | | 11 | | | 6 | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Cadmium (ppm) | | 0.1 | 1.0 | 5.0 | 0.1 | 1.0 | 5.0 | 0.1 | 1.0 | 5.0 |
| 208 Hr. | Alanine | - * | - * | - | - | - | - | + | + | - |
| | Glycine | + | - * | - * | - | - * | - | - | - | - |
| | Osmol. FAA | - | - * | - | - | - | - | - | - | - |
| 376 Hr. | Alanine | - * | - * | D | - | - | + | + * | + * | + |
| | Glycine | - * | - * | D | - | - | + | + | - | + |
| | Osmol. FAA | - * | - * | D | - | - | + | + | + | + |
| 544 Hr. | Alanine | - | - | D | - * | - * | - * | D | CD | CD |
| | Glycine | - | - * | D | - | - | - | D | CD | CD |
| | Osmol. FAA | - | - * | D | - * | - * | - * | D | CD | CD |

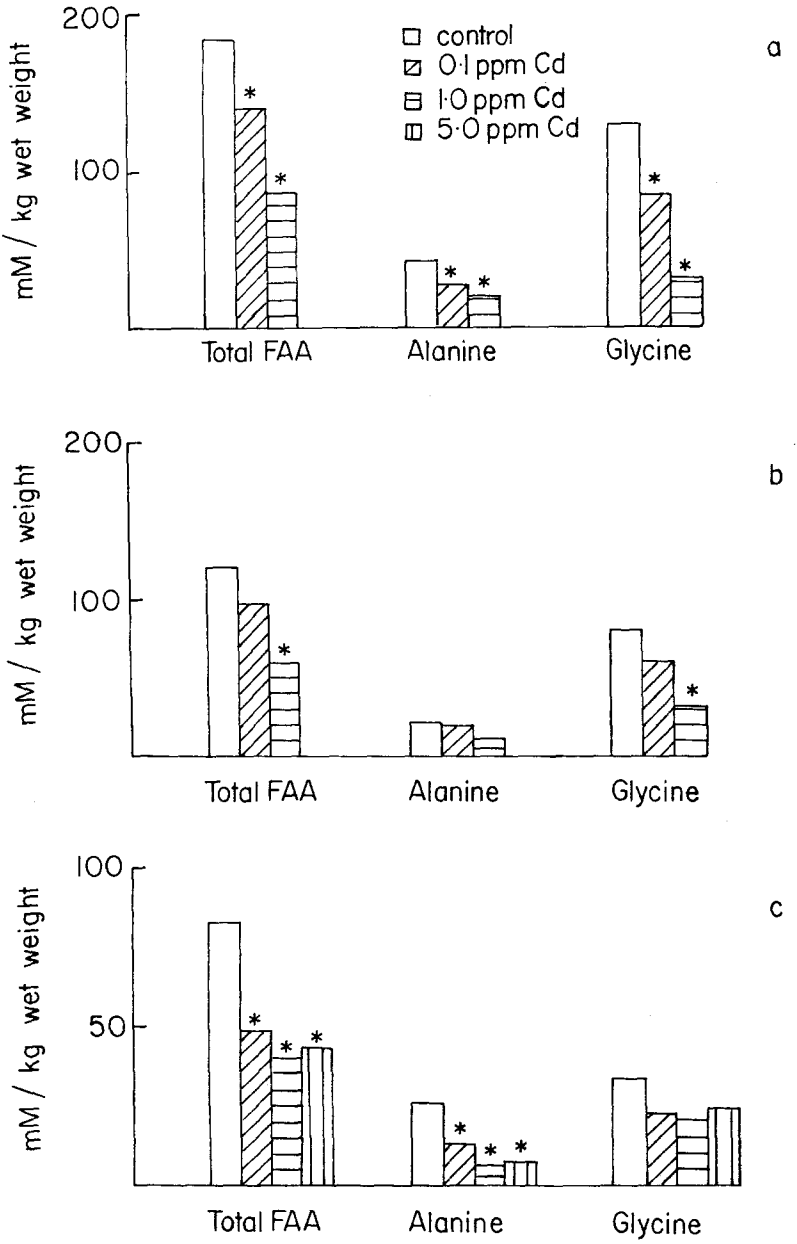


Figure 1. Alanine, glycine, and total FAA levels after exposure to cadmium for 376 hours in 30°/oo (a), 544 hours in 30°/oo (b), and 544 hours in 11°/oo (c). The "*" indicates a significant reduction from the control determined by the Student's t-test with a level of significance of 0.05. The mussels exposed to 5.0 ppm cadmium in 30°/oo salinity were all dead. The cadmium concentrations indicated do not include the background cadmium in Instant Ocean.

TABLE 4

Uptake of cadmium after 544 hours exposure to 0.1 ppm cadmium.

| Salinity (°/oo) | | 30 | | | 11 | |
|---|---|---|---|---|---|---|
| Cadmium (ppm) | $\bar{x}$ | SD | n | $\bar{x}$ | SD | n |
| Gills | 10.86 | 3.25 | 4 | 0.94 | 0.40 | 4 |
| Muscle[a] | 10.51 | 0.81 | 3 | 0.47 | 0.35 | 4 |
| Gonads | 9.07 | 0.58 | 2 | 1.36 | 1.45 | 3 |
| Viscera | 8.98 | 2.64 | 4 | 0.86 | 0.54 | 4 |
| Mantle | 7.61 | 1.59 | 4 | 0.43 | 0.50 | 4 |

[a]does not include the posterior adductor muscle

It is not clear how cadmium present in concentrations below the saturation level of the detoxication system can cause statistically significant changes in adductor muscle FAA concentrations. The work on detoxication systems has been done with "soft tissues" (NOEL-LAMBOT 1976; GEORGE and COOMBS 1977). It is not clear if this includes the muscle. It has been suggested (KIMURA et al. 1974) that metallothioneins protect primarily the kidney and not other organs. It is probable that the muscle has no capacity for detoxication perhaps explaining the similarity of effects on FAA due to the three cadmium levels.

In this study, the mussels in the 11°/oo and 6°/oo salinity series did not open their valves for extended periods of time. No byssal threads were formed and the test solutions remained clear for the entire experiment. Mussels exposed to 11°/oo salinity 0.1 ppm cadmium accumulated much less cadmium after 544 hours than mussels exposed to the same cadmium concentration in 30°/oo salinity (Table 4). These results are probably due to valve closure in response to a stressful environment.

The ecological implications of these results are difficult to assess. Normally, mussels under isosmotic conditions do not lose FAA. The loss of FAA from mussels exposed to cadmium in normal salinity water is a potentially serious effect. If the loss is not reversible, the mussels could become dehydrated and mortality could result. However, Mytilus edulis in all levels of the intertidal zone accumulate cadmium (PHILLIPS 1976). Whether or not the cadmium is bound by metallothionein and thus detoxified is unknown. Perhaps M. edulis may be capable of developing a tolerance to low levels of cadmium over long periods of time.

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