Bull. Environ. Contam. Toxicol. (1986) 36:146–149

Environmental Contamination and Toxicology

Absence of Histopathological Response to Cadmium in Gill and Digestive Diverticula of the Mussel, *Mytilus edulis*

A. S. Giraud,[1] L. K. Webster,[1] J. G. Fabris,[2] L. C. Collett,[2] and N. D. Yeomans[1]*

[1]University of Melbourne, Department of Medicine, Austin Hospital, Heidelberg, Victoria, 3084 and [2]Marine Science Laboratories, P.O. Box 114, Queenscliff, Victoria, 3225 Australia

The blue mussel (Mytilus edulis) has been proposed for use as a sentinel organism to monitor the effects of marine pollution (Goldberg et al., 1978). Recently, there has been interest in quantifying histopathological changes in mussel tissues, as one indicator of pollution-induced stress (Bayne et al., 1980).

Cadmium is a common and toxic aquatic pollutant (Flick et al., 1971; Ahsanullah, 1976; Babich and Stotzky, 1978; Arnott and Ahsanullah, 1979). Bivalves accumulate large amounts of this metal in their tissues (Lake, 1979; Hammond, 1981), particularly in the gill (Carpene and George, 1981) and the digestive diverticula (George and Coombs, 1977). Gill and digestive diverticula have also been shown to be major sites of cadmium detoxification (George and Pirie, 1979). In these same tissues, histopathological changes have been demonstrated after exposure to crude oil (Lowe et al., 1982) and to an oil dispersant (Webster et al., unpublished observations). However, whether similar morphological changes are induced by heavy metals, such as cadmium, is not known.

In this study, we have assessed the cellular effects of sub-lethal concentrations of cadmium on the gill and digestive diverticula of Mytilus.

MATERIALS AND METHODS

Adult mussels were collected at a site in Westernport, Victoria. They were acclimated without food for 6 days, in tanks supplied with continuously flowing, aerated seawater, maintained at 18±1°C.

The toxicant was prepared by dissolving cadmium chloride in seawater, and was delivered to the test tanks using a system previously described by Ahsanullah and Palmer (1980). A total of 240 mussels were distributed randomly among 3 tanks (each of

*Correspondence and reprint author.

60 l capacity), with cadmium concentrations of less than 0.1 µg/l (control), 35 µg/l, and 350 µg/l. After 2 weeks, 40 mussels were removed from each tank. Five randomly selected mussels from each of these subsamples were prepared for histopathological examination and 10 randomly selected mussels were analyzed for Cd using the method of Harris et al. (1979). The tank volume and toxicant flow rates were then halved, and the experiment was continued for a further 2 weeks, after which an additional 45 mussels were prepared as above.

Mussels were reacclimated in aerated seawater for 16-20 h, then opened and flooded with fresh fixative (2% glutaraldehyde, 2.5% formaldehyde, 1.0% picric acid in 0.1 M sodium cacodylate, pH=7.3). Gill and digestive diverticula were dissected out, diced into cubes of 1mm side in a pool of fixative, then fixed for 2h at room temperature. After post-fixing in 1% OsO_4 for 1h, the tissue was washed in 0.1 M cacodylate buffer, dehydrated and embedded in epoxy resin (Araldite). One µm sections of tissue blocks were stained with periodic acid-Schiff (PAS) or by the PAS-Bowie method (Moxey and Yeomans, 1976).

Sections were assessed as follows, with the observer blinded to the origin of the slides. In gill, mucous (PAS-positive) cells were counted in PAS-stained transverse sections of randomly selected gill filaments (ctenidia) from each animal. Digestive diverticula sections were stained with PAS-Bowie. Cell heights of 24 tubule cells per mussel were measured in randomly selected tubules using an eyepiece micrometer.

Statistical comparisons were made by applying the Kruskal-Wallis nonparametric analysis of variance .

RESULTS AND DISCUSSION

The light-microscopic morphology of the gill and digestive diverticula in control mussels appeared normal. The normal structure of these tissues has been well described elsewhere (Owen, 1972; Langton, 1975; Webster et al., 1984).

The cadmium content of mussels prior to the experiment was 0.35±0.37 µg/g soft tissue weight (mean ± SD). After 2 and 4 weeks, cadmium levels in mussels exposed to 35 µg/l Cd were 27±9 and 60±22 µg/l respectively; in mussels exposed to 350 µg/l, the corresponding values were 216±93 and 382±72 µg/g.

In mussels treated with cadmium, neither the number of gill mucous cells nor the heights of digestive diverticula tubule cells were significantly different, compared with controls, at 2 or 4 weeks (Fig. 1).

In this study, we have demonstrated that chronic exposure of mussels to high cadmium levels has no significant effect on the

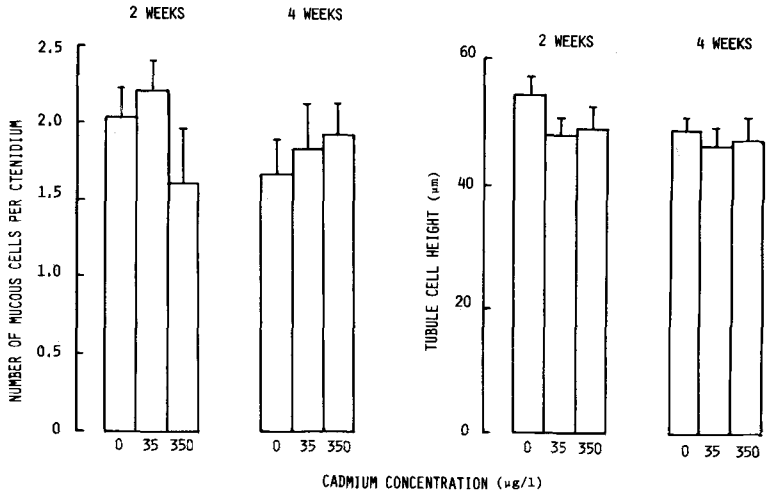

Figure 1. Digestive diverticula cell heights and number of gill mucous cells per ctenidium after 2 and 4 weeks exposure to different cadmium concentrations. N=5. Mean ± S.E. The differences between means are not significant.

number of gill mucous cells, nor on the height of the digestive diverticula tubule cells. This is in contrast to the alterations in these parameters after exposure to oil (Lowe et al., 1981) and an oil dispersant (Webster et al., 1984). Although we postulated that the ability of gill and digestive diverticula to concentrate cadmium might make them particularly sensitive to cadmium toxicity, the content of metal-complexing metalothioniens in these tissues (George and Pirie, 1979) might in fact help to prevent cellular changes from taking place. It remains to be seen whether cadmium or other toxic heavy metals are able to induce histopathological changes in other mussel tissues which lack metalothioniens.

We conclude that, unlike the case with crude oil and dispersants, the value of histopathological indices in assessing the extent and significance of metal ion contamination of marine waters is yet to be demonstrated.

Acknowledgements. This work was carried out with the support of the Environmental Studies Division, Ministry for Conservation, Victoria, Australia. It is not an official policy statement of the Ministry for Conservation.

REFERENCES

Ahsanullah M (1976) Acute toxicity of cadmium and zinc to seven invertebrate species from Western Port, Victoria. Aust J Mar Freshwater Res 27, 187-96

Ahsanullah M, Palmer DH (1980) Acute toxicity of selenium to three species of marine invertebrates, with notes on a continuous-flow test system. Aust J Mar Freshwater Res 31: 795-802

Arnott GH, Ahsanullah M (1979) Acute toxicity of copper, cadmium and zinc to three species of marine copepod. Aust J Mar Freshwater Res 30: 63-71

Babich H, Stotzky G (1978) Effects of cadmium on the biota: influence of environmental factors. Adv Appl Microbiol 23: 55-117

Bayne BL, Brown DA, Harrison F, Yevich PD (1980) The International Mussel Watch. National Academy of Science, Washington

Flick DF, Kraybill HF, Dimitroff J M (1971) Toxic effects of cadmium: a review. Environ Res 4: 71-85

George SG, Coombs TL (1977) The effects of chelating agents on the uptake and accumulation of cadmium by Mytilus edulis. Marine Biology 39: 261-8

George SG, Pirie BJS (1979) The occurrence of cadmium in subcellular particles in the kidney of the marine mussel, Mytilus edulis, exposed to cadmium. The use of electron microprobe analysis. Biochem Biophys Acta 580: 234-44

Goldberg ED, Parker PL, Bowen VT, Risebrough RW, Farrington JW, Robertson W, Harvey G, Scheider E, Martin JH, Gamble E (1978) The mussel watch. Environmental Conservation 5: 101-25

Hammond LS (1981) A review and evaluation of Australian and New Zealand data on Mytilus edulis L. relevant to use of the species as a biological indicator of environmental pollution by heavy metals. Publication No 318 in the Environmental Studies Series, Ministry for Conservation, Melbourne, Victoria

Harris JE, Fabris GJ, Statham PJ, Tawfik F (1979) Biochemistry of selected heavy metals in Western Port, Victoria, and use of invertebrates as indicators with emphasis on Mytilus edulis planulatus. Aust J Mar Freshwater Res 30: 159-78

Lake PS (1979) Accumulation of cadmium in aquatic animals. Chem Aust 46: 26-9

Langton RW (1975) Synchrony in the digestive diverticula of Mytilus edulis L. J Mar Biol Assoc U.K. 55: 221-9

Lowe DM, Moore MN, Clarke KR (1982) Effects of oil on digestive cells in mussels: quantitative alterations in cellular and lysosomal structure. Aquat Toxicol. 1: 213-226

Moxey PE, Yeomans ND (1976) Identification of cell types in semi-thin epoxy sections of gastric fundic mucosa. J Histochem Cytochem 24: 755-6

Owen G (1972) Lysosomes, peroxisomes and bivalves. Sci Prog Oxf 60: 299-318

Received November 30, 1984; accepted February 22, 1985.