

Alteration of the immune response of the common marine mussel *Mytilus edulis* resulting from exposure to cadmium

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ABSTRACT: The common marine mussel *Mytilus edulis* was used in laboratory-controlled experiments to investigate the effects of exposure to cadmium on immune capability. Haemolymph was extracted from mussels exposed for 7 d to 40 and 400 $\mu\text{g l}^{-1}$ of cadmium, and assays were carried out to investigate the potential for immune response. The assays included total and differential haemocyte counts, the release of intracellular and extracellular reactive oxygen metabolites, phagocytosis, release of degradative enzymes and uptake of neutral red. Cadmium at 400 $\mu\text{g l}^{-1}$ resulted in significantly enhanced numbers of circulating haemocytes and increased uptake of neutral red. No alteration was demonstrated in proportions of circulating haemocyte types, phagocytosis or production of reactive oxygen metabolites. The release of degradative enzymes during phagocytosis was found to be significantly suppressed at the 40 $\mu\text{g l}^{-1}$ cadmium concentration. Assays to determine the immune function may provide a sensitive measure of the health status of an individual or population under pollutant stress. However the variability in effects produced in different aspects of immune function emphasises the need for a multi-assay approach to monitoring pollution. The observed effects on immunocompetence, at environmentally realistic levels of contaminant, support the view that a relationship exists between pollution and immunomodulation in aquatic organisms.

KEY WORDS: Mussel · Immunocompetence · Haemolymph · Cadmium

INTRODUCTION

In recent years a great deal of effort has been directed towards elucidating the possible correlation between environmental pollutants and stress-related disease conditions in animals (Sindermann 1993). Bivalve molluscs, including the marine mussel *Mytilus edulis*, have been postulated as ideal indicator organisms for assessing levels of environmental pollution, as they are ubiquitous, sedentary filter-feeders inhabiting coastal and estuarine areas. They filter large volumes of seawater and may therefore concentrate contaminants, up to a factor of 10^5 , within their tissues (Widdows & Donkin 1992). Metals usually occur in a highly soluble form in seawater, and therefore, their uptake and accumulation in filter-feeding bivalves are usually proportional to the concentration in the environment (Livingstone & Pipe 1992).

Cadmium is used principally in the production of stabilisers and pigments in plastic and in the electroplating industry and is also released as a by-product from other anthropogenic activities including mining, metal industries and agriculture. The total input of cadmium to the world oceans is estimated to be approximately 8000 t yr^{-1} , about half of which results from human activity. There is evidence that cadmium levels in the sea and particularly in estuarine sediments may be increasing (Clark 1989).

Contaminants may exert toxic effects on an organism either by direct action on specific body tissues or more generally, by influencing homeostatic mechanisms, such as the immune system. Direct toxic effects resulting in pathological alteration of tissues have been observed in marine species, associated with exposure to cadmium at concentrations as low as 0.001 ppm. The pathological changes recorded have been reviewed by Gardner (1993) and include malformation of the gemmosclere, melanisation particularly

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of gill tissue and cuticle and necrosis of kidney, digestive diverticula and reproductive tissue. The occurrence of neoplastic lesions has also been observed (Gardner 1993).

Mussels have an open circulatory system which is continually exposed to fluctuating environmental factors including contaminants. Investigation of sublethal, toxic effects of contaminants on the immune system may therefore represent a sensitive and comprehensive measure of general health status, reflecting the degree of pollutant stress. Immune defence in mussels is comprised of cell-mediated and humoral mechanisms, in which the haemocytes play a key role (Cheng 1981). Antigenic challenge stimulates migration of haemocytes, followed by phagocytosis and intracellular degradation of the pathogen by means of lytic enzymes (Pipe 1990a) or the production of highly reactive oxygen metabolites (Pipe 1992). Degradation may also occur extracellularly following degranulation of the haemocytes.

Immunomodulation resulting from cadmium exposure has been demonstrated previously in laboratory-controlled experiments using the bivalve *Crassostrea virginica* (Cheng 1988a, b, Larson et al. 1989, Anderson et al. 1992a). The aim of the present study was to investigate the effects of exposure to cadmium on a range of aspects of the immune function of *Mytilus edulis*. Parameters measured concentrated on mechanisms employed by the mussel for destruction of invading pathogens and included changes in the number and character of circulating haemocytes, intracellular and extracellular superoxide radical production, release of lysosomal enzymes, uptake of neutral red and phagocytic activity.

MATERIALS AND METHODS

Experimental mussels. *Mytilus edulis* (60 to 75 mm shell length) were collected at low tide from Exmouth Estuary, Devon, UK. The estuary is in an area with minimal industrial input and therefore is relatively clean. Fifty mussels were allocated to each of 3 polypropylene tanks (26 × 12 × 18 cm) containing 1 l of aerated, filtered seawater at 15°C and were left overnight to acclimatize.

Cadmium exposure. The seawater was changed each day for 7 d, after which the tanks were dosed from a stock solution of cadmium chloride dissolved in seawater, giving final cadmium concentrations of 0, 40 and 400 µg l⁻¹. The mussels were fed 300 ml of mixed algal solution per tank twice weekly, 1 h prior to changing the water.

Protein quantification. In most microplate assays carried out, each haemolymph sample was pipetted into a duplicate microplate and analysed for protein

content using a bicinchoninic acid protein assay (Pierce Chem. Co.). The protein was solubilised using 1% CHAPS at 37°C for 30 min (Pierce Chem. Co.), bovine serum albumen was used as a standard and readings were obtained on the microplate reader at 550 nm. All results were then expressed relative to haemocyte protein content.

Assays. Assays were carried out after 7 d of exposure. Haemolymph was extracted from the posterior adductor muscle (from 6 to 8 mussels per treatment) immediately before each assay, using a 2 ml syringe fitted with a 21 gauge needle.

Total and differential counts: Haemolymph samples were withdrawn into an equal volume of Baker's formal calcium, containing 2% sodium chloride. The samples were mixed and allowed to fix. Total cell counts were performed using a haemocytometer (improved Neubauer). Blood cells were prepared for differential counts using a cytocentrifuge (Shandon); 250 µl of haemolymph was spun at 170 × g onto glass microscope slides. Cells were then post-fixed in methanol for 10 min, stained with Wright's stain (Gurr, BDH) for 2 min, rinsed in water, air dried and mounted in Canada balsam. Relative numbers of eosinophils and basophils were calculated by counting 300 blood cells from each mussel.

Superoxide generation: Haemolymph was extracted into an equal volume of 0.05 M Tris-HCl buffer, pH 8.2, containing 2% NaCl. Aliquots of 100 µl of each were pipetted into microplate wells in replicates of 8.

Cytochrome-C reduction: Cytochrome-C solution (100 µl aliquots of 80 µM cyt-C in Tris-HCl buffer) was added to 4 wells for each of 6 samples and cytochrome-C solution containing 300 units ml⁻¹ of superoxide dismutase (SOD) (Sigma Chem. Co.) added to the remaining 4 wells. A stimulant (such as phorbol myristate acetate) was not found to be necessary as previous studies have determined that experimental manipulation is sufficient to activate the cells and elicit superoxide generation (Pipe 1992, Coles et al. 1994). Wells containing cells in buffer only and cytochrome-C solution without cells were prepared as negative controls. The optical density (OD) was read immediately at 20°C on an Anthos HTII microplate reader, using a narrow band-width, 550 nm filter and a kinetics programme, taking readings every 30 s over a 20 min period. The results were expressed as unit changes in OD over 20 min mg⁻¹ protein.

Nitroblue tetrazolium salt (NBT) reduction: NBT solution (2 mg ml⁻¹) dissolved in Tris-HCl buffer (100 µl aliquots) was added to 4 replicate microplate wells of each of 6 haemolymph samples. NBT solution containing 300 units ml⁻¹ of SOD was added to the remaining 4 wells. Blood cells in buffer only and NBT solution without cells were used as blank control samples. The

plates, wrapped in foil, were incubated for 20 min at 10°C, after which the cells were centrifuged at $120 \times g$ for 10 min, washed twice in buffer and fixed for 10 min in methanol. The plates were then centrifuged at $300 \times g$ for a further 10 min and the cells allowed to air dry. After 5 further washes in 50% methanol, the cells were solubilised in 140 μ l of dimethyl sulfoxide (DMSO) (Sigma Chem. Co.), followed by 120 μ l of 2 M KOH. The plates were read using a 620 nm filter and the results expressed as OD mg^{-1} protein.

Phagocytosis: A method was developed to assess the total phagocytic uptake capability of the blood cells using a microplate reader. Haemolymph was withdrawn into an equal volume of anticoagulant buffer (0.05 M Tris-HCl, pH 7.6, containing 2% glucose, 2% NaCl and 0.5% EDTA). Aliquots of 50 μ l of each sample were pipetted into 12 replicate microplate wells. An equal volume of neutral-red-stained, heat-stabilised, zymosan suspension (Sigma Chem. Co.), containing 1×10^8 particles ml^{-1} in anticoagulant buffer, was added to each well. Aliquots of zymosan suspension in anticoagulant buffer alone were used as controls and aliquots of heat-killed cells in zymosan suspension were used as blanks. The microplates were incubated for 25 min at 10°C, and the process was stopped by adding 100 μ l aliquots of Baker's formol calcium, containing 2% NaCl, to duplicate wells. The cells were allowed to fix for 10 min, after which they were centrifuged at $70 \times g$ for 5 min and washed several times in buffer. Suspensions of known zymosan concentrations were aliquoted (50 μ l) to duplicate wells just prior to the last centrifugation to provide a standard curve. Acetic acid (1%) in 50% ethanol (100 μ l) was added to each well to solubilise the neutral red and the plate allowed to stand for 5 min. The OD was then read at 550 nm and the results expressed as zymosan concentration phagocytosed mg^{-1} of haemocyte protein.

Release of degradative enzymes: The release of N-acetyl- β -D-glucosaminidase (NAG) was measured. Haemolymph was withdrawn into anticoagulant buffer and 50 μ l aliquots were pipetted into 8 replicate microplate wells. Zymosan suspensions (50 μ l) of 0.0125, 0.025 and 0.05% in anticoagulant buffer were added to the wells in duplicate and buffer alone added to the remaining 2 wells. The plates were left to incubate for 1 h at 10°C, after which they were centrifuged at $300 \times g$ for 10 min. Supernatant (50 μ l) was then removed and placed into corresponding wells of a clean plate. To each was added 50 μ l of the substrate, P-nitrophenyl-N-acetyl- β -D-glucosaminide (dissolved in 2.5 ml of dimethyl sulfoxide and diluted to 15 mM in anticoagulant buffer) and 50 μ l of anticoagulant buffer. The plates were incubated for a further 20 min at 20°C, after which 100 μ l of stopping buffer (0.4 M glycine buffer, pH 10.4) was added. A standard curve was pre-

pared using P-nitrophenol and wells containing all solutions but without blood cells were used as blanks. The OD was read at 340 nm. Enzyme release was found to be optimal upon stimulation with 0.05% zymosan and therefore the results were expressed as μ M of enzyme released mg^{-1} of haemocyte protein at this concentration of zymosan.

Uptake of neutral red: Haemolymph was withdrawn into an equal volume of anticoagulant buffer and 100 μ l aliquots of each sample pipetted into 3 replicate microplate wells. Aliquots (10 μ l) of 0.33% neutral red (Sigma Chem. Co.) solution in phosphate buffered saline (PBS) containing 2% NaCl were added to each well and the plate incubated for 1 h at 10°C. The cells were then centrifuged at $200 \times g$ for 5 min and washed twice in buffer. Aliquots (100 μ l) of 1% acetic acid in 50% ethanol were added to all wells. The plates were incubated, covered with foil, for 15 min at 20°C and then read at 550 nm. Protein analysis was carried out on duplicate wells and the results expressed as OD mg^{-1} protein.

Cadmium analysis. Lysosomal compartments, particularly those in the digestive gland, represent the major site of metal accumulation in bivalve molluscs (Livingstone & Pipe 1992). The digestive gland was removed from 6 mussels from each treatment and placed into PBS containing 2% NaCl. The tissues were then digested in concentrated nitric acid at 20 ml g^{-1} dry wt, heat-evaporated and made up in 10% hydrochloric acid. Analysis for cadmium content was then carried out by flame atomic absorption spectrophotometry, using deuterium arc background correction for NaCl (Bryan et al. 1985).

Statistical analysis. Results of all assays were analysed statistically using 1- or 2-way analysis of variance (Systat). Mean values are expressed \pm 1 SE.

RESULTS

Cadmium analysis

Analysis of the cadmium content of the digestive gland tissue of mussels demonstrated increasing tissue levels with increasing cadmium administered (Table 1).

Table 1. *Mytilus edulis*. Levels of cadmium found in the digestive gland tissue of mussels exposed to cadmium for 7 d

Exposure level ($\mu\text{g l}^{-1}$)	Level in tissue ($\mu\text{g g}^{-1}$ dry wt)
0	2.87
40	7.37
400	99.81

Assays

No mortalities were produced in either control or cadmium exposed mussels.

Total and differential cell counts

The effect of cadmium on the total number of circulating haemocytes in *Mytilus edulis* is shown in Fig. 1. Exposure to $400 \mu\text{g l}^{-1}$ of cadmium resulted in a significant increase in haemocyte number to 13×10^6 cells ml^{-1} , as compared to 8×10^6 cells ml^{-1} in the control mussels ($p = 0.021$) and 5.7×10^6 cells ml^{-1} in the mussels exposed to $40 \mu\text{g l}^{-1}$ of cadmium ($p = 0.001$).

The differential counts identified basophilic and eosinophilic cells. The basophilic haemocytes ranged from small hyalinocytes to cells containing numerous small granules, the eosinophilic haemocytes contained large granules (Pipe 1990b). The effect of cadmium exposure on the percentage of circulating eosinophilic to basophilic cells is shown in Table 2. Exposure did not result in any significant fluctuations in proportion of blood cell types.

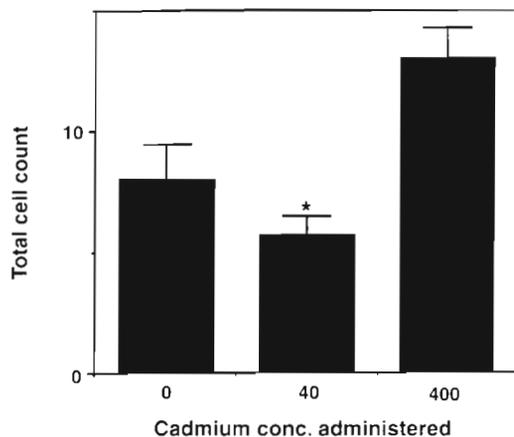


Fig. 1. *Mytilus edulis*. Total number of circulating haemocytes ($\times 10^6$ cells ml^{-1}) in the haemolymph of mussels exposed to different concentrations of cadmium ($\mu\text{g l}^{-1}$) for 7 d. *Significant ($p < 0.05$)

Superoxide generation

The results of cadmium exposure on generation of superoxide radicals by *Mytilus edulis* haemocytes are shown in Table 2. Both cytochrome-C reduction, measuring extracellular superoxide release, and NBT reduction, measuring intracellular superoxide release, showed no significant variation between mussels from control or exposed tanks.

Table 2. *Mytilus edulis*. Effects of 7 d cadmium exposure on proportions of circulating cell types, extracellular and intracellular superoxide production and phagocytic activity by haemocytes. Extracellular superoxide is measured by the reduction of cytochrome-C (change in OD over 20 min mg^{-1} haemocyte protein), intracellular superoxide by reduction of nitroblue tetrazolium salt (OD mg^{-1} haemocyte protein) and phagocytosis by uptake of neutral-red stained zymosan (no. of zymosan particles $\times 10^{10}$ mg^{-1} haemocyte protein)

	Exposure level ($\mu\text{g l}^{-1}$)		
	$0 \mu\text{g l}^{-1}$	$40 \mu\text{g l}^{-1}$	$400 \mu\text{g l}^{-1}$
Eosinophils: basophils (%)	49.51 ± 1.9	43.57 ± 3.1	52.48 ± 3.0
Extracellular superoxide	0.094 ± 0.009	0.075 ± 0.008	0.079 ± 0.01
Intracellular superoxide	0.138 ± 0.048	0.095 ± 0.029	0.167 ± 0.045
Phagocytosis	2.57 ± 0.45	2.89 ± 0.35	1.85 ± 0.48

Phagocytosis

Table 2 shows the effect of cadmium exposure on the phagocytic activity of mussel haemocytes following 25 min incubation. Cadmium at $400 \mu\text{g l}^{-1}$ resulted in a significant ($p = 0.016$) decrease in phagocytosis of zymosan as compared to mussels exposed to $40 \mu\text{g l}^{-1}$ cadmium but this decrease in phagocytosis was not statistically significant when compared with control mussels.

Release of degradative enzymes

Fig. 2 shows the effect of cadmium exposure on zymosan-stimulated release of N-acetyl- β -D-glucosaminidase by mussel haemocytes. Cadmium at $40 \mu\text{g l}^{-1}$ produced a significant ($p = 0.01$) suppression in the release of NAG as compared to both the control mussels and those exposed to $400 \mu\text{g l}^{-1}$ cadmium. The higher cadmium level appeared to stimulate NAG release but this was not significantly different from the control value.

Uptake of neutral red

Fig. 3 shows the effect of cadmium exposure on the uptake of neutral red by mussel blood cells. Cadmium at $400 \mu\text{g l}^{-1}$ resulted in a significant ($p = 0.028$) increase in uptake of the dye by the haemocytes as compared to control mussels. Cadmium at $40 \mu\text{g l}^{-1}$ resulted in a slight but non-significant ($p = 0.430$) increase in uptake by the haemocytes.

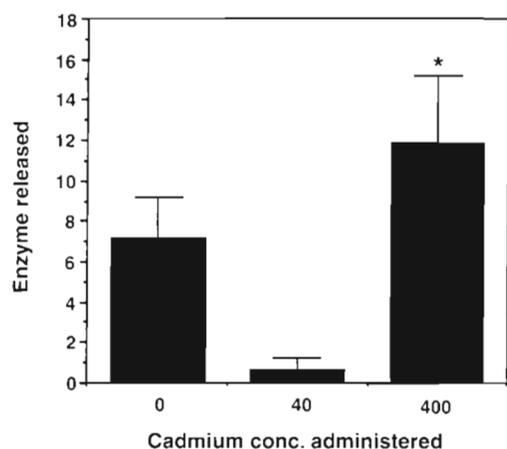


Fig. 2. *Mytilus edulis*. Zymosan-stimulated release of N-acetyl- β -D-glucosaminidase (μM of enzyme mg^{-1} of haemocyte protein) from haemocytes of mussels exposed to different concentrations of cadmium ($\mu\text{g l}^{-1}$) for 7 d. *Significant ($p < 0.05$)

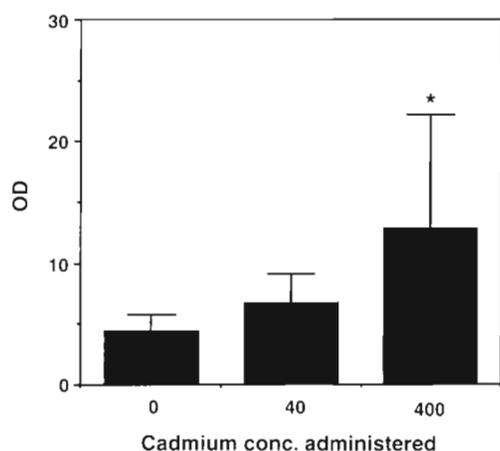


Fig. 3. *Mytilus edulis*. Uptake of neutral red (OD mg^{-1} of haemocyte protein) by haemocytes of mussels exposed to different concentrations of cadmium ($\mu\text{g l}^{-1}$) for 7 d. *Significant ($p < 0.05$)

DISCUSSION

Immunomodulation associated with exposure to cadmium has been observed previously in a range of species. Altered haematological parameters and effects on immune function have been found in both fish (Zelikoff 1993) and bivalves (Anderson 1993).

In the present study, exposure to cadmium over a 7 d period was carried out at environmentally realistic levels of 40 and 400 $\mu\text{g ml}^{-1}$. Cadmium levels of more than 50 $\mu\text{g l}^{-1}$ (Bryan & Langston 1992) and 210 $\mu\text{g l}^{-1}$ (W. J. Langston pers. comm.) have been recorded at Restronguet Creek, Cornwall, UK. These experimental

levels have been shown to produce elevated levels of cadmium in the digestive gland tissue of the mussel and to affect significantly several aspects of immune function. Exposure to cadmium at 400 $\mu\text{g l}^{-1}$ resulted in a significant increase in the total number of circulating haemocytes whilst not affecting the relative proportions of eosinophilic to basophilic cell types. Elevation in total blood cell numbers seems to be a common response to environmental stressors. Exposure of *Mytilus edulis* to fluoranthene (Coles et al. 1994) or exposure to phenol or stress by temperature manipulation (Renwantz 1990) resulted in increased total blood cell counts, of which the last was found to be reversible on withdrawal of the stressor. Exposure of *Crassostrea virginica* to cadmium (Cheng 1988a) or to a protozoan parasite (Anderson et al. 1992b) also stimulated circulating blood cell numbers and a reversible increase in cells was found to occur 72 h after exposure of 2 clam species, *Ruditapes philippinarum* and *R. decussatus*, to pathogenic bacteria (Oubella et al. 1993). These relatively rapid, reversible responses suggest that increased total numbers of circulating haemocytes occurs by stimulation of migration of the cells from tissues, rather than by blood cell proliferation. This may or may not result in altered relative proportions of haemocyte types depending on the distribution within the tissues of blood cell types in a particular individual or species. The diverse nature of the stimulants suggests that this is a general response to an environmental stressor rather than a reaction to a specific challenge.

The present results show that cadmium exposure also significantly affected some aspects of haemocyte function. The production of superoxide radicals by the haemocytes is postulated to play an important role in intra- and extracellular killing of invasive pathogens in invertebrates (Adema et al. 1991) as well as vertebrates. Alteration of the chemiluminescent response, measuring the production of reactive oxygen metabolites by haemocytes, has been found in association with high levels of polynuclear aromatic hydrocarbons (PAHs) in several fish species (Weeks et al. 1990a, b) and in association with cadmium exposure in the carp (Elasser et al. 1986). Exposure to cadmium *in vitro* has been found to affect haemocyte chemiluminescent activity in the oyster, *Crassostrea virginica* (Larson et al. 1989, Anderson et al. 1992a). However, this occurred at relatively high environmental levels of dissolved cadmium (10 to 320 ppm); *in vivo* exposure, up to 0.25 ppm, did not result in significant alterations in chemiluminescence (Anderson et al. 1992a). The production of superoxide radicals by immune cells was not found to be significantly altered by exposure to the environmentally realistic levels of cadmium used in this study.

Phagocytosis prior to intracellular degradation of invasive antigens comprises an integral part of cellular defence in organisms. Environmental stressors are postulated to be capable of reducing immunocompetence by altering the phagocytic activity of haemocytes. Depressed phagocytic activity by haemocytes of various species of fish have been found associated with high levels of PAHs (Weeks et al. 1989, Weeks et al. 1990a, b, Seeley & Weeks-Perkins 1991). In molluscs, phenol exposure was found to depress phagocytosis by haemocytes of *Merceneria merceneria* (Fries & Tripp 1980) whereas long-term, *in vitro* exposure to benzo(a)pyrene, pentachlorophenol and hexachlorobenzene produced a slight increase in phagocytic activity in this clam (Anderson 1981). Heavy metals have also been found to alter phagocytic activity. Exposure *in vivo* to 1 ppm cobalt, 5 ppm chromium, 5 ppm copper, 1 and 5 ppm iron, 0.1 ppm mercury and 5 ppm selenium resulted in increased phagocytosis by the haemocytes of *Crassostrea virginica* and 0.5 to 5 ppm mercury significantly decreased phagocytosis, whereas 1 and 5 ppm cadmium produced no effect (Cheng & Sullivan 1984). Exposure of *C. virginica*, for 1 to 2 wk *in vivo*, to 1 ppm copper and 1 ppm cadmium resulted in an inhibitory effect by the former and stimulation of phagocytosis by the latter (Cheng 1988b). In the present study, 7 d exposure of *Mytilus edulis* to 40 $\mu\text{g l}^{-1}$ cadmium produced a stimulation of phagocytosis by the haemocytes, whereas 400 $\mu\text{g l}^{-1}$ resulted in a slight inhibition. However, although significantly different to each other, these alterations are not significantly different to the control values when comparing 6 mussels. Further experiments using more mussels would possibly demonstrate alteration in phagocytic activity due to cadmium exposure by reducing the effects of the variability observed between individuals.

The uptake of neutral red by mussel haemocytes may occur by pinocytosis or passive diffusion across the cell membrane. Neutral red is a cationic dye which accumulates in the lysosomal compartment of cells. Alteration in its uptake may reflect damage to the plasma membrane of the blood cells or alternatively, may represent changes in the volume of the lysosomal compartment. In the present study, uptake of neutral red was significantly increased on exposure to 400 $\mu\text{g l}^{-1}$ cadmium. Depressed phagocytosis together with increased neutral red uptake by macrophages was also demonstrated in studies on several fish species captured from highly polluted areas (Weeks et al. 1989). This effect may demonstrate the ability of certain contaminants, including cadmium, to alter the permeability of blood cell membranes as postulated by Pickwell & Steinert (1984).

The potential for microbicidal activity by release of degradative enzymes during phagocytosis is another

important method of cellular, immune defence in invertebrates. The presence of a range of lysosomal enzymes has been demonstrated previously in the granules of *Mytilus edulis* haemocytes (Pipe 1990a). Pickwell & Steinert (1984), working on *M. edulis*, found an increase in release of degradative enzymes into haemolymph under cupric ion stress and postulated that this resulted from disruption of lysosomal membranes. Cheng (1989), however, found stimulation of β -glucuronidase activity by copper exposure in *Crassostrea virginica* but inhibition, by both copper and cadmium, of the release of this enzyme and acid phosphatase into the haemolymph. More recently, Coles et al. (1994) demonstrated inhibition by fluoranthene of the release of chymotrypsin-like enzyme by blood cells of *M. edulis*. This study demonstrates that exposure of mussels to cadmium at 40 $\mu\text{g l}^{-1}$ may result in a significant inhibition of the release of the glycolytic enzyme NAG into the haemolymph. Cadmium at 400 $\mu\text{g l}^{-1}$ resulted in an increase in enzyme release although not to a significant degree. Contrasting effects produced by lower and higher levels of contaminant are commonly demonstrated and may signify that a threshold level of contaminant may be an important concept when assessing immunomodulation (Larson et al. 1989). The variation in effects on enzyme release by different stressors suggests that these may result from a specific interaction between the chemical and its target, rather than representing a general stress response.

Overall, this study demonstrates that short-term cadmium exposure, at environmentally realistic levels, may affect various aspects of immune function in *Mytilus edulis*, with the possible consequence of an impaired defence capability and increased disease susceptibility of an individual or population. Indeed, the lower concentration of 40 $\mu\text{g l}^{-1}$ produced a more significant alteration in the release of NAG from the blood cells. The variation found in the extent and nature of the response to a contaminant and the difference in the responses observed as compared to those in different species emphasise the need for a multi-assay and multi-species approach to the study of effects on immunocompetence. The assays used, when combined, may therefore represent a sensitive means of testing for environmental stressors and their possible effects on disease susceptibility in aquatic organisms. Further research is needed to test for the potentially more harmful effects on disease susceptibility of long-term exposure to contaminants.

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