EFFECTS OF CADMIUM IONS ON THE ULTRASTRUCTURE OF THE GILL CELLS OF THE BROWN SHRIMP *CRANGON CRANGON* (L.) (DECAPODA, CARIDEA)

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INTRODUCTION

Metal ions are serious pollutants especially in aquatic environment since they can be incorporated into food chains and concentrated by aquatic organisms to a level which affects their physiological state (Bryan, 1971).

The gills are one of the most permeable regions of the body and are the sites of respiration and of transport system involved in osmoregulation (Bielawski, 1971; Quinn & Lane, 1966). Physiological, histological and ultrastructural studies have shown that heavy metal ions interfere with respiration and osmoregulation by disrupting the structure of the gill cells in fish and crustaceans (Baker, 1969; Eisler & Gardner, 1973; Jones, 1975). Few studies, however, have been carried out on the correlation between the morphological changes which occur in the gill cells after exposure to metal ions and a possible change in the function of the different organelles (Bubel, 1976; Nimmo et al., 1977).

The brown shrimp *Crangon crangon* (L.) is an ecologically important species in British waters. It was of interest, therefore, to examine the toxic effects that cadmium, in the shape of CdCl₂.2½ H₂O, has on the ultrastructure of the gill cells of this species and relate them with functional differences in the organelles.

MATERIALS AND METHODS

Specimens of *C. crangon* were collected at Oxwich Bay, Swansea, U.K., in February, March and April 1978, at low tide and kept alive at 15°C and in 30‰ circulating sea water. Some of the collected specimens were berried females. Under these conditions the specimens were kept for a week before use. All experimental animals were fed with chopped fish fingers on alternate days during this holding period, after which standard-sized individuals were placed in artificial sea water of 30‰ salinity at 15°C. The artificial sea water was made up by dissolving “Tropical Marine” salts, distributed by Shirley Aquatics Ltd., Solihul, England, in glass distilled water. A range of concentra-
tions of cadmium ions varying between 50 and 5 ppm showed to have an effect on the ultrastructure of the gill cells of *C. crangon*. Therefore groups of five specimens were placed in 50, 25 and 5 ppm of cadmium ions. A fourth group of active specimens was placed in clean sea water as control. After 20 hours, which is the lethal time for 50% of specimens placed in 50 ppm cadmium at 15°C (Papathanassiou, unpubl.), alive individuals were removed from all groups and placed in 5% cacodylate buffered glutaraldehyde with 0.17 M sucrose. The gills from specimens of each group were fixed for 1 h in the above solution at 0-4°C. These were then washed in several changes of buffered sodium cacodylate with sucrose added followed by post fixation in 1% osmium tetroxide solution for 1 h at 0-4°C. After dehydration in graded cold acetone the material was embedded in TAAB embedding resin. Sections with gold or silver interference colours were obtained using a Huxley Mark I Ultramikrotome and were mounted on coated grids. The tissues were then double stained in 30% uranyl acetate (30 min.) followed by lead citrate (10 min.) and viewed in a Corinth AEI Electron Microscope.

A multi-purpose analysis was performed to test the relative volumes of mitochondria and the surface to volume ratio of their cristae in specimens from the three concentrations and from the controls.

Electron micrographs were enlarged to a final magnification of $25 \times 10^3$ and analysed using the double-lattice test system (Weibel et al., 1966) in order to test the relative volumes of mitochondria.

Individual mitochondria were enlarged to a final magnification of $102.6 \times 10^3$ and analysed using the multi-purpose test system consisting of 100 points enclosing 50 short test lines (Weibel et al., 1966) for testing the surface to volume ratio of their cristae. The data obtained for the morphometric analysis were analysed by applying the t-test.

**RESULTS**

The gill lamellae in *C. crangon* consist of an outer cuticle beneath which are the epithelial cells and a central haemocoele. Figures 1.1, 1.2, 2.1 and 2.2 show diagrammatic and epithelial cell in the gills of *C. crangon* in untreated sea water and after exposure to 5, 25 and 50 ppm of cadmium respectively.

In control specimens the cuticle is composed of an outer layer of moderate electron density about 0.06 μm thick and an inner layer of 0.37-0.40 μm thick, consisting of three distinct zones (fig. 3.5). The outer and inner zones are 0.018 μm and 0.015 μm thick respectively and are homogeneously moderate electron dense. These zones are separated by an electron translucent middle zone about 0.34 μm thick. In the epithelial cells there are abundant mitochondria many of which are associated with the infoldings of the basal cell membrane (fig. 3.1). Each cell has a nucleus containing dispersed chromatin near the periphery (fig. 3.2) and is connected to the next one by septate desmosomes with a well