Bull. Environ. Contam. Toxicol. (1986) 36:192–198

Environmental Contamination and Toxicology

Cadmium Accumulation and Ultrastructural Alterations in Oogenesis of the Prawn *Palaemon serratus* (Pennant)

E. Papathanassiou*

Department of Zoology, University College of Swansea, United Kingdom

Cadmium exists in the marine environment in trace quantities (approximately 0.53 mμmol/L in British waters) largely in ion form (Wright 1977a). However in high concentrations it is very toxic with lethal effects (Bryan 1971; Ahsanullah 1976). In marine crustaceans, an accumulation of cadmium ions by different tissues, under experimental conditions, has been studied, especially in relation to salinity and temperature (O'Hara 1973; Hutscheson 1974; Wright 1977a; 1977b; Nimmo et al 1977).

The gills, hepatopancreas and the carapace were reported as the major sites of localization of cadmium after short term exposure of blue crabs to cadmium solutions (Hutscheson 1974). A similar pattern of accumulation occurs in the molluscs, the gonad of which is one of the tissues where mercury ions are accumulated, when the animals are exposed to mercury solutions (Cunningham and Tripp 1975).

The reproductive cycle of Palaemon serratus (Pennant), an ecologically important species, was studied by Forster (1951) in Plymouth and by Cole (1958) in North Wales. Recently Papathanassiou and King (1984) have made ultrastructural studies of the oogenesis of P. serratus. The present study was undertaken to determine the effects of cadmium on the process of oogenesis of the common prawn and the levels of cadmium in the gonads after exposure to cadmium ions. This is important since it will give an indirect indication on how pollutants affect different tissues especially at the ultrastructural level.

MATERIALS AND METHODS

Specimens of P. serratus were collected from rocky pools in Oxwich bay, Swansea, U.K., and they were then kept in running sea water of 30‰ salinity at ambient temperature (15±3 °C) for at least a week before use.

After this period, groups of 20 active individuals were placed in

* Present address: Institute of Oceanographic and Fisheries Research Ag. Kosmas, Hellinikon, Athens, GR 166 04, GREECE

crystallizing dishes containing 1 L of artificial sea water at 15°C for 48 h. Specimens were then placed in three cadmium concentrations, 5, 25 and 50 ppm and in clean sea water to act as control. Four replicate dishes were used for each concentration. The sea water was made up by dissolving "Tropical Marine" salts, distributed by Shirley Aquatics Ltd., Solihul, England, in glass-distilled water. Observations were made every 6 h, when dead individuals were removed. The solutions were renewed after 24 h.

After 44 h, which is the lethal time for 50% of the individuals placed in 50 ppm cadmium ions at 15°C (Papathanassiou 1984), alive, active specimens were removed from the dishes and washed in glass-distilled water to remove any medium from the external part of the animals.

A group of ten individuals was removed from each concentration. This number of animals was proved to be sufficient to give readings on the spectrophotometer. Thus, forty animals (four groups of ten) were examined from each treatment. The gonads were dissected from each individual and dried at 105°C for 24 h. These tissue samples were then weighed to the nearest 0.1 mg (dry weight less than 10 mg) and digested in 5 mL of 5:1 Nitric:Perchloric acid at 120°C, under continuous vacuum, making certain that temperature would not exceed the 120°C. The determination of the accumulation of cadmium was made by using a Corning-Eel 240 Mark II Flame Atomic Absorption Spectrophotometer (Wright 1977a).

For the electron microscope, live specimens were placed in 5% glutaraldehyde solution at pH 7.4 for 2 h. They 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 siver interference colors were obtained using a Huxley Mark I Ultramicrotome and were mounted on coated copper grids. They were then double stained in 30% uranyl acetate (30 min) followed by lead citrate (10 min) and viewed in a Corinth AEI Electron Microscope.

RESULTS AND DISCUSSION

After 44 h exposure the gonads accumulated cadmium ions in proportion to the surrounding medium. The accumulation was significantly higher than in control animals ($p < 0.001$) (Table 1). Control animals accumulated a small amount of cadmium in the gonad. In the control animals the ovaries contained 1.75 ppm cadmium, with two out of four groups having accumulated vitrually none. After exposure to 5 ppm cadmium, 11.8 ppm cadmium was accumulated in these tissues (575% increase over the controls), while after exposure to 25 ppm and 50 ppm cadmium the accumulation levels were 34.7 ppm (1883% increase over the controls) and 48.3 ppm (2660 % increase over the controls) respectively (Table 1). The high vari-

Table 1. Mean values of accumulated cadmium/dry weight in the ovaries of P. serratus after exposure to cadmium ions for 44 h. Values in parenthesis represent % increase over the controls.

Concentrations (ppm)	Cadmium accumulated in the gonad(ppm ± SD)	
Control	1.75 ± 1.15	
5	11.80 ± 8.90	(574.3)
25	34.70 ± 6.80	(1882.8)
50	48.30 ± 8.70	(2660.0)

ability, which is observed in Table 1, was mainly due to the different rate of accumulation depending on the different maturation stage of the eggs within the ovaries. These high concentration values found in the gonads of P. serratus could be a result of ions which pass from the haemolymph to the gonad. In support to this postulation is the fact that cadmium has been found entering the haemolymph of Carcinus maenas L., when placed in solutions containing high cadmium concentrations (Wright 1977b). However the total amount of cadmium in the gonads after 44 h exposure to 50 ppm of cadmium was approximately half of that in the gills (Papathanassiou and King 1983).

The process of oogenesis at the ultrastructural level , in P. serratus has been studied by Papathanassiou and King (1984). After exposure for 44 h in 5 ppm and 25 ppm cadmium ions, no morphological alterations in the oocytes could be observed. Figures 1.1 and 1.2 show the cytoplasm in a developing oocyte of a control specimen. The only change that was observed in oocytes of specimens exposed to 50 ppm cadmium was in the mitochondria. They change shape and their cristae become swollen, especially during the late stages of vitellogenesis (Figs. 2.1, 2.5). Within the mitochondria sometimes dielectronic material was observed, without a limiting membrane (Fig. 2.1). All these features indicate that the mitochondria do not perform their normal function and therefore the energy level declines. Similar studies that have been done in the gill cells of crustaceans have shown great differences at the ultrastructural level in specimens treated with heavy metal ions. These differences were atributed to the effect that these ions have on enzymic and ATP-ase activity, absorption and transportation of salts, active ion uptake and protein synthesis (Bubel 1976; Papathanassiou and King 1983). The form of mitochondria varies, being either dumbbell shaped, cup-shaped, spherical or oval (Fig. 2.4). Cup-shaped mitochondria were observed during vitellogenesis in association with unaltered parts of oöplasm which contains abundant free ribosomes and eventually engulf part of the oöplasm or other mitochondria (Figs. 2.2, 2.3). During the later stages of vitellogenesis the mitochondrial cristae are swollen, but there is no evidence of mitochondria engulfing cytoplasm (Fig. 2.6). Ratcliffe and King

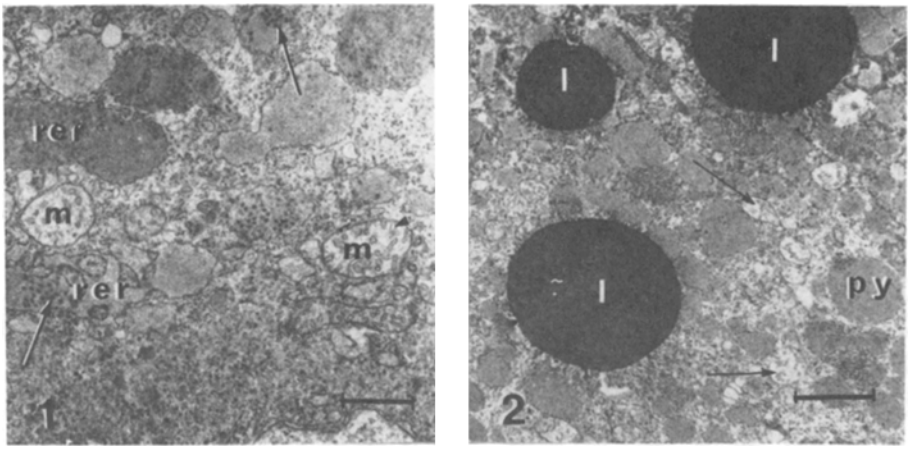


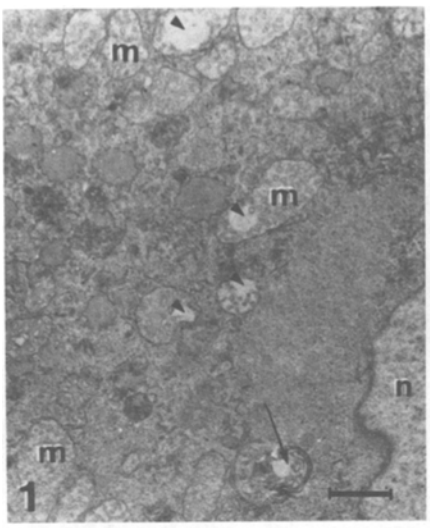
Figure 1.1. Electron micrograph of a control specimen, showing a vitellogenic oöcyte. Note the shape of the mitochondria (m) and their cristae (arrowhead). Rough endoplasmic reticulum (rer) contain intracisternal granules (arrows). Scale bar 1 µm.

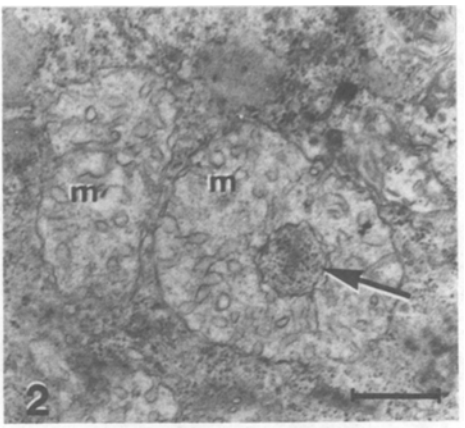
Figure 1.2. Electron micrograph of a control specimen, showing the cytoplasm of a vitellogenic oöcyte. Note the mitochondria (arrows), the protein yolk body (py) and the lipid droplets (l). Scale bar 2.5 µm.

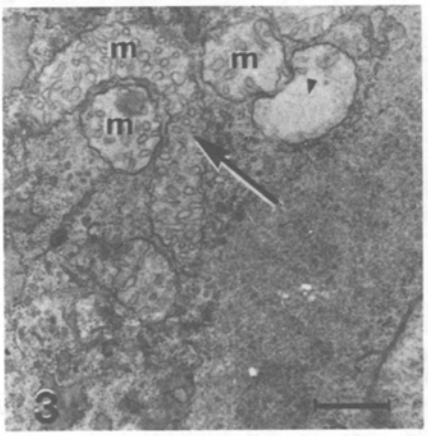
(1969), suggested that similar transformation of mitochondria in the acid gland of starved Nasonia vitripennis (Walker) (Insecta), is an attempt by the mitochondria either to regain their energy level or help them to extract glycolysable substances from both the surrounding cytoplasm and from their own components.

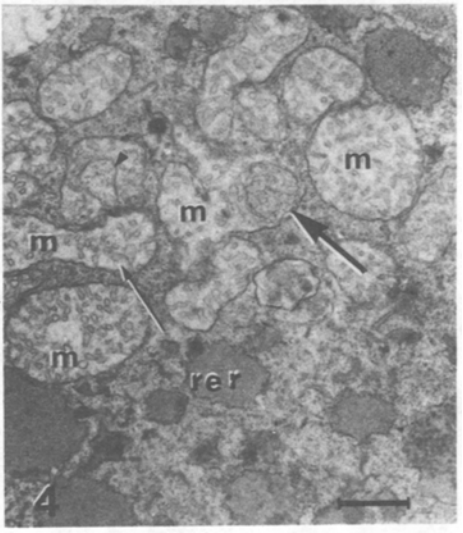
Thus, results show that accumulated cadmium ions affect the mitochondria in the developing oöcyte of P. serratus, and therefore, since mitochondria are involved in the metabolism of the oöcyte, this metabolism is affected. Further studies, however, must be done to show if the mechanism of yolk production is affected by longer exposure to cadmium ions, in solutions which represent the real environmental conditions.

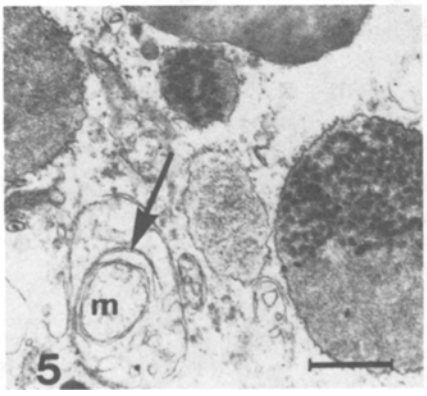
Acknowledgements. I am indebted to Dr. P.E. King for his advice and helpful criticism and to Professor E. W. Knight-Jones, in whose department this work has been carried out. I would also like to thank Dr. M. Fordy for his technical assistance on the Electron Microscope.


m
m
n
m
1


m
m
2


m
m
m
3


m
m
m
m
rer
4


m
5

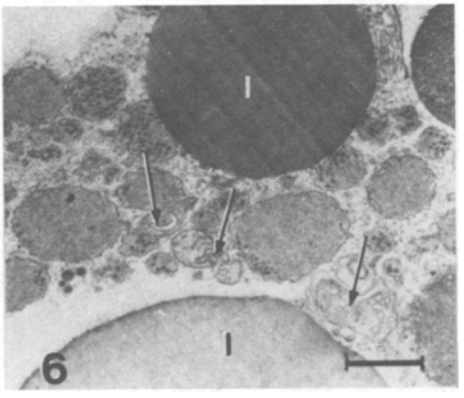

l
l
6

Figure 2.1. Electron micrograph showing a vitellogenic oöcyte after exposure to 50 ppm cadmium. Note the mitochondria (m) containing dielectronic material (arrowheads). Nucleus (n). Scale bar 1 µm.

Figure 2.2. Electron micrograph showing mitochondrion (m) enclosing portions of cytoplasm (arrow) in a vitellogenic oöcyte, after exposure to 50 ppm of cadmium. Scale bar 0.5 µm.

Figure 2.3. Electron micrograph showing mitochondrion (m) engulfing one of its neighbours (arrow) in a vitellogenic oöcyte, after exposure to 50 ppm cadmium. Scale bar 0.7 µm.

Figure 2.4. Electron micrograph showing the different shapes of mitochondria (m) during vitellogenesis, after exposure to 50 ppm cadmium. Note the dumb-bell shaped (arrow), the cup-shaped (small arrowhead) and the spherical mitochondria. Note that one mitochondrion has engulfed portion of the cytoplasm (big arrow). Scale bar 0.8 µm.

Figure 2.5. Electron micrograph showing mitochondrion (m) with digenerate cristae (arrow) during late stages of vitellogenesis, after exposure to 50 ppm cadmium. Scale bar 0.5 µm.

Figure 2.6. Electron micrograph showing mitochondria with swollen cristae (arrows) during late stages of vitellogenesis. Lipid droplets (l). Scale bar 1 µm.

REFERENCES

Ahsanullah M (1976) Acute toxicity of cadmium and zinc to seven invertebrate species from Western Port, Victoria. Aust J Mar Freshwat Res 27:187-196

Bryan GW (1971) The effect of heavy metals (other than Hg) on marine and estuarine organisms. Proc Roy Soc Lond B177:389-410

Bubel A (1976) Histological and electron microscopical observations on the effects of different salinities and heavy metal ions on the gills of Jaera nordmanni (Rathke)(Crustacea, Isopoda). Cell Tiss Res 167:65-95

Cole HA (1958) Notes on the biology of the common prawn Palaemon serratus (Pennant). Fishery Investigations, Ser III, Vol 22(5), HMSO, London

Cunningham PA, Tripp MR (1975) Accumulation, tissue distribution and elimination of $^{203}HgCl_2$ and $CH_3{}^{203}HgCl$ in the tissue of the American oyster, Grassostrea virginica. Mar Biol 31:321-334

Forster GR (1951) The biology of the common prawn, Leander serratus Pennant. J Mar Biol Ass UK 30:333-360

Hutscheson MS (1974) The effect of temerature and salinity on cadmium uptake by the blue crab, Callinectes sapidus. Chesapeake Sci 15:237-241

Nimmo RDW, Lightner VD, Bahner HL (1977) Effects of cadmium on the shrimps, Penaeus duorarum, Palaemonetes pugio and Palaemonetes vulgaris. In: Vernberg FJ,Calabrese A, Thurberg FP, Vernberg WB (eds) Physiological responses of marine invertebrates to pollutants. Academic Press, New York, p 131-183

O'Hara J (1973) The influence of temperature and salinity to fiddler crab Uca pugilator. Fish Bull 71:149-153

Papathanassiou E, King PE (1983) Ultrastructural studies on the gills of Palaemon serratus (Pennant), in relation to cadmium accumulation. Aquat Toxicol 3:273-284

Papathanassiou E (1984) Effects of cadmium and mercury ions on respiration and survival of Palaemon serratus (Pennant). Rev Int Oceanogr Med 72:21-35

Papathanassiou E, King PE (1984) Ultrastructural studies on gametogenesis of the prawn Palaemon serratus (Pennant). I. Oogenesis. Acta Zool 65:17-31

Ratcliffe NA, King PE (1969) Ultrastructural changes in the mitochondria of the acid gland of Nasonia vitripennis (Walker) (Pteromalidae:Hymenoptera) induced by starvation. Z Zellforsch 99:459-468

Wright DA (1977a) The effect of salinity on cadmium uptake by the tissues of the shore crab, Carcinus maenas. J Exp Biol 67:137-146

Wright DA (1977b) The uptake of cadmium into the haemolymph of the shore crab Carcinus maenas : The relationship with copper and other divalent cations. J Exp Biol 67:147-161

Received December 14, 1984; accepted March 9, 1985