Ann. Zool. Fennici 17: 243-247. 1980

Effects of phenol and 4-chlorophenol on ionic regulation in Mesidotea entomon (Crustacea) in brackish water[1]

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Oksama, M. & Kristoffersson, R. 1980: Effects of phenol and 4-chlorophenol on ionic regulation in *Mesidotea entomon* (Crustacea) in brackish water. — Ann. Zool. Fennici 17: 243—247.

The isopod *Mesidotea entomon* (L.) was exposed to various concentrations of phenol (5°C and 10°C) and 4-chlorophenol (5°C) in continuous-flow experiments in brackish water (salinity ca. 6 ‰) and the effects of the two phenols on the ion content of the haemolymph were studied. At phenol concentrations exceeding the LTC (lethal threshold concentration) in this hypo-osmotic medium surviving animals had significantly increased calcium and magnesium concentrations in their haemolymph. Sodium and chloride concentrations were found to be decreased, the changes being steeper at the lower temperature.

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1. Introduction

The toxic effects of several pollutants on aquatic animals have been attributed at least partly to disturbances in osmotic and ionic regulation. *Mesidotea* is an osmoconformer in polyhaline waters and a hyperosmotic regulator in brackish water (Bogucki 1931, Lockwood & Croghan 1957, Croghan & Lockwood 1968, Vinogradov 1973, Lockwood et al. 1976). The more pronounced the difference in ionic concentration between medium and haemolymph, the greater is the energy required to maintain this gradient (Lockwood 1962).

In brackish waters even a small change interfering with osmotic and ionic regulation may seriously affect survival. Heavy metals and chlorinated hydrocarbons have been shown to disrupt the normal osmoregulatory balance in crustaceans capable of maintaining a marked osmotic gradient between the blood and the surrounding medium (Thurberg et al. 1973, Jones 1975, Inman & Lockwood 1977). The disruption is most pronounced at low salinities, which place the greatest demands on the osmoregulatory system. We therefore investigated the effect of phenols on the concentrations of major ions in the haemolymph of *Mesidotea*. The toxicity of phenols to *Mesidotea* under conditions identical to those used here has been reported previously (Oksama & Kristoffersson 1979).

2. Materials and methods

Mesidotea were collected with a bottom trawl from the water area of Tvärminne Zoological Station in June-July at a depth of about 30 m, where the temperature in summer was 4—11°C. Before the tests the isopods were acclimated to 10°C for at least 3 weeks in continuously flowing brackish water in 100-l glass aquaria with washed sand on the bottom. The salinity of the water was approximately 6 ‰. The major ion content was: chloride 94—98 mEq/l, sodium 75.8—82.2 mEq/l, potassium 1.7—1.8 mEq/l, calcium 4.2—4.3 mEq/l and magnesium 17.3—17.8 mEq/l (Kristoffersson et al. 1972). During this acclimation period the animals were fed twice a week with pieces of fresh fish, but feeding was stopped 2 days before the experiments. The experimental design was the same as that described by Oksama & Kristoffersson (1979).

In the continuous-flow experiments the animals were exposed to six different phenol concentrations, ranging from 50 to 400 ppm at 5°C and 10°C, and to five different 4-chlorophenol concentrations, ranging from 20 to 100 ppm at 5°C. A previous study with *Mesidotea* (length 3.8 ± 0.4 cm) (Oksama & Kristoffersson 1979) has shown that the lethal threshold concentration (LTC) for phenol is 101 ppm at 5°C and 86 ppm at 10°C, and for 4-chlorophenol 37 ppm at 5°C. Fifteen large specimens of both

[1] Report No. 631 from Tvärminne Zoological Station, University of Helsinki.

sexes (length 5.1 ± 0.6 cm) were used in this study at each phenol concentration. The toxicity of phenols appears to be the same for animals of different sizes.

Because the moulting cycle affects the ionic regulation of crustaceans (Passano 1960, Hagerman 1973), only animals at the intermoult stage were used in the tests. The concentrations of ions (Na^+, K^+, Mg^{2+}, Ca^{2+}, Cl^-) were determined in haemolymph samples taken from surviving animals that had been exposed to lethal phenol concentrations after the death of 50 % of the animals in each test batch. When sublethal concentrations were tested the haemolymph samples were taken 2 weeks after the start of exposure. The sample (0.05 ml) was taken from the dorsal vein at the level of the fifth dorsal segment, and immediately diluted in 0.1 % lanthanum solution. Sodium, potassium, magnesium and calcium were determined from appropriate dilutions by atomic absorption spectrophotometry (Perking Elmer 305). The chloride content of the haemolymph was determined in samples from animals exposed to phenol concentrations of 180 and 270 ppm. A chloride titrimeter (Aminco) was used.

3. Results

The results of the analyses are depicted in Figs. 1—3.

At sublethal phenol concentrations there were no significant differences in haemolymph ion content between the controls and the animals exposed to phenols. A 5°C rise in temperature had no significant effect on the ion content in control animals. At concentrations above the LTC the amount of sodium was inversely proportional to the phenol concentration from the control level of 241—251 mEq/l to 177—193 mEq/l at 400 ppm phenol concentration. A similar relation was observed for chloride concentration: the mean for the controls was 264 mEq/l, but at 180 ppm phenol only 179 mEq/l, and at 270 ppm 172 mEq/l. At the LTC of 4-chlorophenol there was occasionally a transitory increase in sodium concentration, but above the LTC the amount of sodium decreased, as in the case of pure phenol.

The variation in the K^+ level, within the limits of 7 and 12 mEq/l, was not correlated with the phenol concentrations. It may have been caused by cellular injury due to the sampling method used.

At the highest phenol and 4-chlorophenol concentrations tested calcium showed a 6- to 8-fold increase as compared with the controls. The increase was significant even at the lowest phenol concentrations that were toxic. At the lower temperature the increase in calcium concentrations seemed to be steeper. The control values for calcium were about 19 mEq/l; at the highest phenol concentrations the values were 113—151 mEq/l. The magnesium concentration of the haemolymph, too, increased at lethal phenol concentrations. The control values (21—23 mEq/l) differed significantly from those found in animals exposed to the highest phenol concentrations (41—53 mEq/l).

Although the sodium concentration decreased, the calcium and magnesium concentrations increased so greatly that the sum of the cations was higher in the groups that had been exposed to phenol. In pure brackish water the proportion of sodium is about 80 % of the total cations. At high phenol concentrations the decrease was about 30 %.

4. Discussion

The disruption of ionic balance in the haemolymph may be a reaction to 'environmental stress' induced by phenol or may be associated with the toxic mechanism of phenols, for at concentrations exceeding the LTC the loss of ionic regulatory ability parallels the increasing concentrations.

The study was carried out in natural brackish water, which has a total cation concentration about 200 mEq/l lower than in the haemolymph

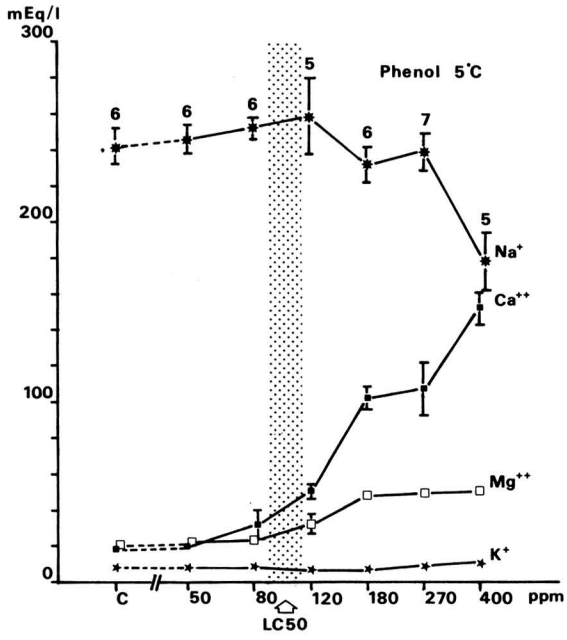

Fig. 1. Haemolymph ion content (mean ± *SE*) in *Mesidotea* exposed to different concentrations of phenol at 5°C. The LTC is 101 ppm, the stippled area being the 95 % confidence limits. The numbers of analyses are shown in the figure.

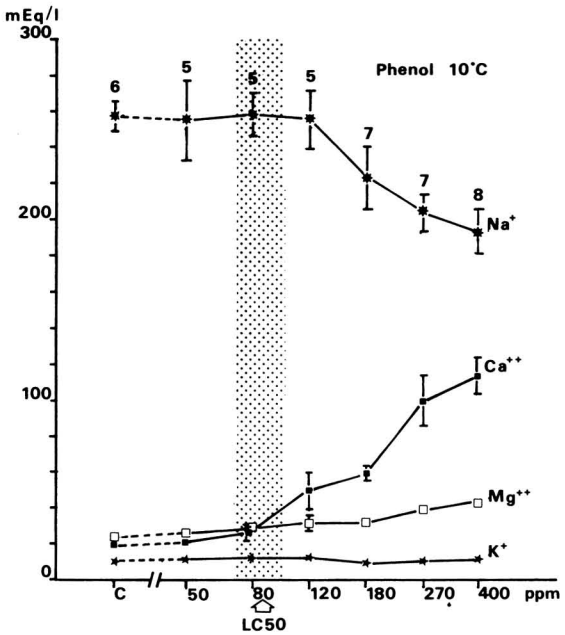

Fig. 2. Haemolymph ion content (mean ± *SE*) in *Mesidotea* exposed to different concentrations of phenol at 10°C. The LTC is 86 ppm, the stippled area being the 95 % confidence limits. The numbers of analyses are shown in the figure.

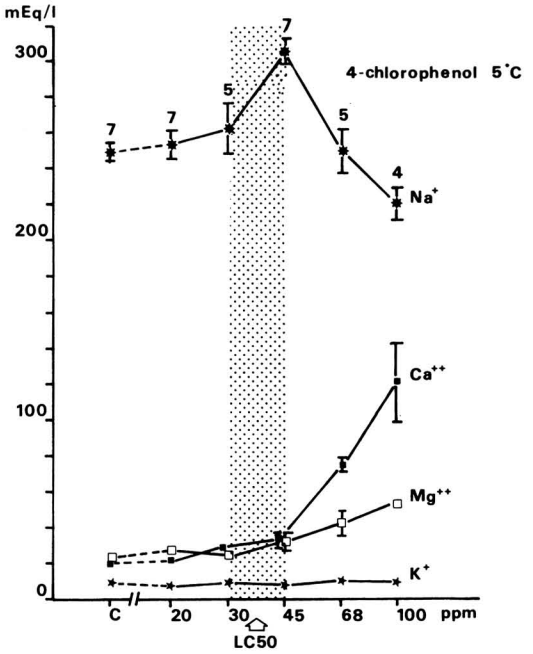

Fig. 3. Haemolymph ion content (mean ± *SE*) in *Mesidotea* exposed to different concentrations of 4-chlorophenol at 5°C. The LTC is 37 ppm, the stippled area being the 95 % confidence limits. The numbers of analyses are shown in the figure.

of *Mesidotea* (cf. Kristoffersson et al. 1972). Na^+ and Cl^- are the ions principally responsible for the osmotic pressure of the haemolymph between moults (Florkin 1960, Potts & Parry 1964). In hyperosmotic regulators exposure to some heavy metals leads to a decrease in the osmotic value of the blood (Thurberg et al. 1973, Jones 1975). The results suggest that exposure to phenols, too, may cause the osmolality of the haemolymph to fall. As the present study showed, however, the amounts of Ca and Mg in the haemolymph increased so greatly that the total cation concentration increased. But since in the haemolymph Ca and Mg are partly bound to proteins their contribution to the maintenance of osmotic balance is reduced. Moreover, among the factors accounting for osmoregulation in crustaceans, the free amino acids both in the cells and in the haemolymph have compensating properties that are of considerable value (Gerard & Gilles 1972, Siebers et al. 1972).

In hyperosmotic regulators toxic substances may also have exactly the opposite effect on the osmotic value of haemolymph. Cadmium, for example, increases the osmotic value of the haemolymph in hyperosmotic regulators (Thurberg et al. 1973). This has been thought to be a result of a selective effect of the toxicant on some specific tissues or organs (cf. Inman & Lockwood 1977). Any substance interfering with the properties of biological membranes will affect ionic regulation.

In crustaceans the metabolism of Ca and Mg is closely linked with the moulting cycle. Between moults the crustaceans living in dilute media store both Ca and Mg in various sites (hepatopancreas, thoracic sterna and/or gastroliths), and these stores are mobilized when needed or replenished when minerals are resorbed from the old cuticle (Passano 1960). Some pollutants have a lytic effect on the hepatopancreas (Couch et al. 1974). This might induce a release of calcium and magnesium. The alterations in the haemolymph ion contents observed in the present study may possibly be related to such damage to the storage tissues.

The decrease in sodium and chloride contents of the haemolymph may be due to an increase in the permeability of the cuticle and/or inhibition of active ion transport. A number of phenols are potent uncouplers of oxidative phosphorylation (Scarpelli 1974), thus inhibiting ATP formation. The permeability of the gills may be changed in a way that increases the diffusion of ions to the hypotonic medium. In fish high phenol concentrations have been shown to induce pathological changes in the respiratory surface of the gills (Waluga 1966, Mitrovic et al. 1968). Therefore, in *Mesidotea*, too, phenols may be assumed to affect the structure of the gill tissue of pleopods.

There is some evidence that blood Mg and behaviour are correlated. The higher the Mg concentration, the less effective is neuromuscular transmission. Another factor influencing tonus and excitability is the Ca/Mg ratio in the blood (Robertson 1960). The temporary increase in the magnesium concentration and especially the change in the Ca/Mg ratio observed during exposure to phenol may contribute to the inhibition of motor function, and consequently to general activity. Lindström & Lindström (1980) have shown that even at sublethal concentrations of phenol (1—30 ppm) and 4-chlorophenol (1—10 ppm) the effect on the swimming activity of *Pontoporeia affinis* was either momentarily activating and inhibiting or from the very beginning inhibiting. The low activity then persisted in clean water despite a certain degree of recovery.

The bivalve *Tellina tenuis* shows good recovery from exposure to phenol (Stirling 1975). Lockwood & Croghan (1957) reported that *Mesidotea* moribund in fresh water recovered well when returned to brackish water, although in their experiments the haemolymph chloride concentration in fresh water had decreased to a level lower than that reported here. Preliminary tests with *Mesidotea* exposed to phenol indicate fairly good recovery as regards both survival and ionic balance. From the results of this study it is not yet possible to decide whether the changes in ionic concentration observed are a special reaction to phenols or only a general response to environmental stress.

Acknowledgements. The authors want to thank Mrs. Jean Margaret Perttunen, B.Sc. (Hons.), for correcting the English text. This study was supported by a grant from the Emil Aaltonen Foundation.

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Received 16. IV. 1980
Printed 31. XII. 1980