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Effects of low concentrations of heavy metals on the bivalve Macoma balthica[1]

Jón Eldon, Marketta Pekkarinen & Rolf Kristoffersson

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Permanent and transitory changes in burrowing activity and damage to tissues caused by short-term exposure of *Macoma balthica* to low concentrations of different heavy metals are described. Small specimens (8—12 mm) of *M. balthica* were exposed for 24 h to Hg, Cd, Cu, Zn, Pb, Ni, and Co at concentrations ranging from 0.01 to 100 ppm at 12°C and 6 ‰ salinity. Hg and Cu reduced burrowing activity at concentrations as low as 0.05 and 0.2 ppm, respectively. In the case of Co, 20 ppm was needed to produce a significant decrease. The range of concentrations from the lowest affecting burrowing activity to that producing complete inhibition was narrow for Cd, Cu and Ni and wider for the other metals. Recovery of burrowing activity in animals exposed to Hg, Cd and Ni was poor. Mortality was high in animals exposed to Hg and Cd.

With all the metals tested, exposure caused losses of siphon parts in up to 30 % of the animals. Histological examination of the damaged siphons revealed such lesions as separation of the outer and inner epithelia, constriction and swelling of the siphon walls and deterioration of the well-organized structure of the siphon in the control specimens. The failure of the microvillous epithelium to ward off the effects of environmental agents is discussed.

Jón Eldon, Dept. of Clinical Biochemistry, University Hospital (Landspitalium), Reykjavik, Iceland.

Marketta Pekkarinen and Rolf Kristoffersson, Division of Physiology, Department of Zoology, University of Helsinki, Arkadiankatu 7, SF-00100 Helsinki 10.

1. Introduction

The assessment and description of sublethal effects of toxicants on organisms, rather than determination of LC_{50} values or contamination analyses, is attracting increasing interest even among researchers working with marine organisms (cf. Vernberg et al. 1977a, 1977b). Studies have focused mainly on Hg and Cd, but Cu, Zn and Pb have also come into the limelight. Less attention has been paid to the other heavy metals, probably because they have been shown to be less poisonous to organisms or less abundant in the environment.

Macoma balthica (L.) has proved to be a suitable test animal. Its life history is well known, it is usually abundant in its habitat, easy to sample all the year round and easy to maintain under laboratory conditions (Yonge 1949, Segerstråle 1960, 1965, Beukema et al. 1977, Eldon & Kristoffersson 1978). The burrowing behaviour of *M. balthica* is a delicate indicator of toxicants such as dissolved heavy metals in the ambient water (Eldon & Kristoffersson 1978), or in contaminated sediments (McGreer 1979).

The aim of this study was to assess the effects of low concentrations of different heavy metals in sea water on the burrowing behaviour of *Macoma balthica* and on its recovery after short-term exposure. It soon became evident that heavy metals affected the siphons of *Macoma*. This prompted histological examination and measure-

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ment of the general effects of heavy metals on the siphons. The results are outlined here.

2. Material and methods

The *M. balthica* material was collected in October-December (1977 and 1978) from a permanent sampling station off Tvärminne Zoological Station. Animals with shells 8—12 mm long were used, as earlier tests had shown that animals of this size category burrow more actively than larger specimens (Eldon & Kristoffersson 1978). The animals were kept in the laboratory in plastic tanks (40 × 24 × 12 cm) with a 4-cm layer of sifted (1 mm sieve) and thoroughly washed dune sand on the bottom and over this a layer of continuously flowing (450 ml/min) natural, aerated brackish water (6°C, 6 ‰ S) ca. 5 cm deep. The experiments were carried out (in January-February) at 12°C, a temperature to which the animals were acclimated for 6 days before the tests. The metals tested were Cd ($CdSO_4 + 8H_2O$), Hg ($HgCl_2$), Cu ($CuSO_4 + 5H_2O$), Zn ($ZnSO_4 + 7H_2O$), Pb ($Pb(NO_3)_2$), Ni (NiCl) and Co ($CoCl_2 + 6H_2O$). All the salts used were of analytical grade. Exposure concentrations varied from 0.01 to 100 ppm. The animals were exposed to the metals in plastic aquaria (25 × 20 × 12 cm) with no sand on the bottom for 24 h. After exposure they were transferred to identical aquaria with clean water and sand on the bottom. Burrowing activity was recorded by counting how many animals had burrowed at successive time intervals (Eldon & Kristoffersson 1978). The animals were left in the clean aquaria and mortality was observed for 15 days. After this the burrowing activity of surviving specimens was tested anew. The results of these tests are expressed (see Fig. 1) as a 'percentage inhibition effect' (p.i.e.) of the exposed specimens as compared with the control specimens. The values were calculated from the formula

$$\text{'p.i.e.'} = 100\,\Sigma n/\Sigma m - 100$$

where n = % of exposed animals that burrowed at each observation time during the test, and m = corresponding value for control animals. The number of observations in each pair of experiments was the same.

Pieces of siphons that had broken off during the 24-h or longer exposure of the animals to the metals were collected for histological examination. These pieces as well as parts of siphons that had been exposed, but had not broken off, were fixed in Bouin's fluid. As controls, siphons of unexposed animals were fixed at various phases of extension. The samples were taken through butanol, embedded in paraffin and sectioned at 5—7 μm. The following staining methods were used: Masson — Gomori (chromotrope — fast green; Gray 1954), Masson — Goldner (Burck 1969), Alcian blue — PAS — Mayer's haematoxylin (Mowry, 1963, after Pearse 1968) and Crossmon's haematoxylin — acid fuchsin — orange — light green (Romeis 1968).

For EM studies pieces of intact siphons and feet were prefixed for 1.5 h in 4 % glutaraldehyde in a 0.1 M phosphate buffer (pH 7.2) and postfixed for 1.5 h in 1.5 % OsO_4 in the same buffer. The pieces were embedded in EPON 812 (Ladd Research Industries, Inc.), sectioned with a Porter-Blum MT_1 microtome, and stained with lead citrate (Reynolds 1963). The sections were examined with a Zeiss EM 9 S electron microscope.

3. Results

Hg affected the burrowing activity of *M. balthica* at concentrations as low as 0.05 ppm (Fig. 1, solid circles). Exposure to 1 ppm Hg for 24 h completely inhibited all burrowing activity. Keeping the animals in a clean aquarium for 15 days did not restore the burrowing activity to the control value (Fig. 1, open circles). Of the animals that had been exposed to 1 ppm Hg for 24 h, 70 % were dead within 15 days. The corresponding value for 0.5 ppm was 15 %.

Exposure to 0.5 ppm Cd for 24 h had a stimulating effect on burrowing activity. Exposure to 1 ppm caused a reduction of activity of about 30 % as compared with controls and exposure to 2 ppm led to almost total inhibition. After 15 days in a clean aquarium few animals showed signs of recovery, and about 85 % of those that had been exposed to 2 ppm were dead. The corresponding figure for 1 ppm was 45 %. None of the animals died after exposure to 0.5 ppm Cd.

Exposure to 0.2 ppm Cu inhibited burrowing activity in about 40 % of the animals and 2 ppm inhibited it completely. The recovery of the animals exposed to Cu was good. None of the animals died after exposure to Cu within the concentration range of 0.1—2 ppm.

Exposure to 0.5 and 1.0 ppm Zn stimulated burrowing activity, whereas concentrations in excess of 2 ppm inhibited it. 50 ppm inhibited burrowing activity completely. Recovery was good up to 20 ppm: of the animals exposed to 20 ppm 25 % succumbed within 15 days, and of the animals likewise exposed to 50 ppm 65 %.

After exposure to 0.5 ppm Pb burrowing activity was slightly reduced and addition of Pb to 20 ppm caused a reduction of 75 %. This concentration exceeds the solubility of lead nitrate in sea water (Schulz-Baldes 1972). Recovery was good and no animal died.

Exposure to Ni began to have an effect at a concentration of 5 ppm, and 20 ppm led to complete inhibition of burrowing activity. The animals did not recover well from exposure to Ni, but none died.

Reduction in burrowing activity after exposure to Co was significant only at concentrations as high as 20 ppm. Exposure to 100 ppm led to complete inhibition. The animals recovered well after exposures to Co, and none died.

Exposures lasting 24 h to all the metals tested caused damage to the siphons, and up to 30 % of the animals lost parts of their siphons (Fig. 2). In the control animals breaking off of siphons was never seen. The lowest concentrations causing

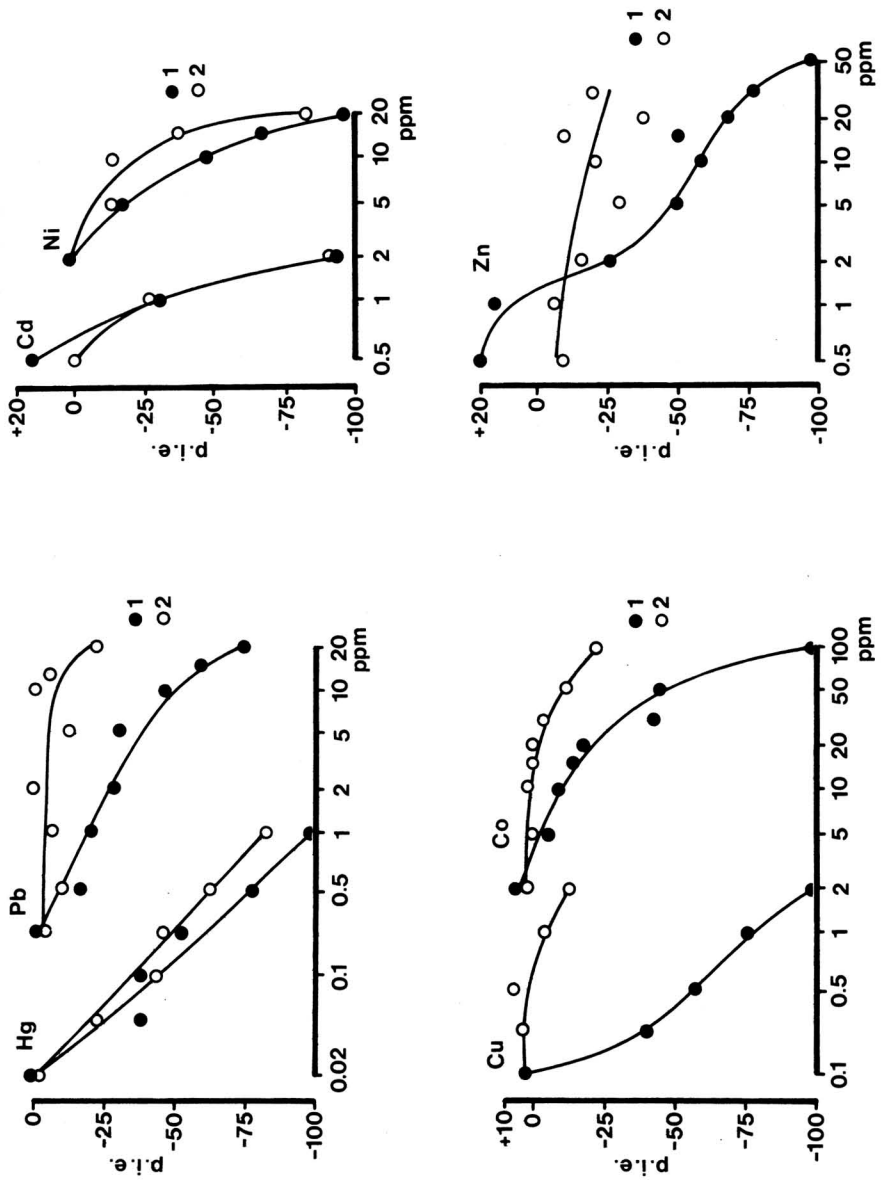

Fig. 1. Percentage inhibition effect ('p.i.e.', see text) in *M. balthica* exposed to various concentrations of Hg, Cd, Cu, Zn, Pb, Ni and Co. Solid circles (1) after exposure for 24 h. Open circles (2) after recovery for 15 days in clean aquaria (12°C, 6‰ S; 0 = control value). On the abscissa ppm is a log 10 scale. The curves were drawn by free hand.

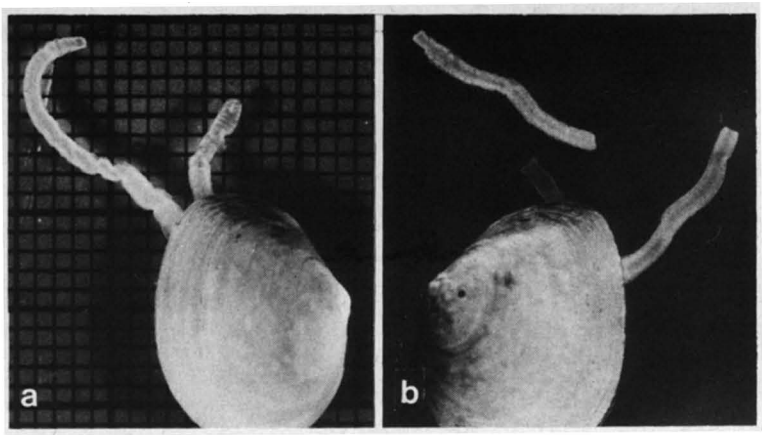

Fig. 2. Two individuals of *M. balthica* that had been exposed to 5 ppm Cd for 24 h. In a) the siphons are wrinkled. In b) part of one siphon has broken off. Millimetre paper provides a scale.

this injury were 0.05 ppm for Hg, 0.5 ppm for Cu, 1 ppm for Cd, 1 ppm for Ni, 5 ppm for Pb, 5 ppm for Zn and 30 ppm for Co.

The siphons of *Macoma balthica* (Fig. 3) are similar in structure to those of *Scrobicularia plana* (Yonge 1949). There are circular, longitudinal and radial sets of muscle fibres. Six nerves run along the length of the siphons. Numerous haemocoelic lumina, one or two of which are often larger than the rest, run longitudinally between the inner and outer bundles of longitudinal muscle fibres and between the sets of radial muscle fibres. The areas of these lumina vary considerably from one preparation to another. The inner and outer epithelia of the siphons are covered with microvilli, and occasionally there are small tufts of cilia (Fig. 4). It should be mentioned here that the foot is also covered with microvilli. When the siphons contract, the epithelia, the outer longitudinal muscle fibres and even the nerves become puckered. The inhalant and exhalant siphons are very similar in structure.

The siphonal walls of the animals that had been exposed to metals often showed different stages of wrinkling, constriction and swelling (cf. Figs. 2a and 5). Other common changes were vacuolization and loosening of the inner and/or outer epithelium, derangement of the muscle fibres, and pyknotic nuclei (Figs. 5c and 6). The diameter of the siphons was reduced, particularly on exposure to Co at 30 ppm and 50 ppm. The siphons of many animals that had been exposed to Zn were heavily contracted. Siphons exposed to Zn usually showed little if any damage. Exposure to Hg and Cu, even at low concentrations, did the greatest histopathological damage. Even at 0.5 ppm of Hg and Cu, the siphons lost their epithelia, and their muscular tissue was in poor condition (Figs. 6b and 6c). Individual variation was seen.

In some individuals exposure to Cd, Co, Pb and Zn led to an increase in the number of polymorphonuclear or polynuclear haemocytes. In normal animals such cells were rare.

4. Discussion

When ranked according to effective threshold concentration, i.e., to the lowest concentration that has detectable effects (increases or decreases) on the burrowing activity of *Macoma balthica,* the metals follow the order: Hg < Cu < Cd < Zn < Pb < Ni < Co. This sequence agrees with the findings and rankings presented by Brown & Ashanullah (1971), Stebbing (1976) and Calabrese et al. (1977).

The 'p.i.e.' curves show, by their span and shape, how increasing concentrations of a metal affect the burrowing activity of *M. balthica.* Metals with narrow 'p.i.e.' curves affect burrowing activity within considerably narrower limits at concentrations exceeding the threshold value (Cd, Ni). The effect of metals with broad curves increases more slowly with increasing concentration (e.g. Zn). When the metals are ranked according to the increasing span of their 'p.i.e.' curves, the order is somewhat different: Cd < Ni, Cu < Hg, Co < Zn (Pb).

Hg inhibited burrowing activity at lower concentrations than the other metals tested, the animals recovered poorly from exposure, and mortality during the recovery period was high. Sublethal concentrations of Hg have been found to decrease the metabolic rate and swimming

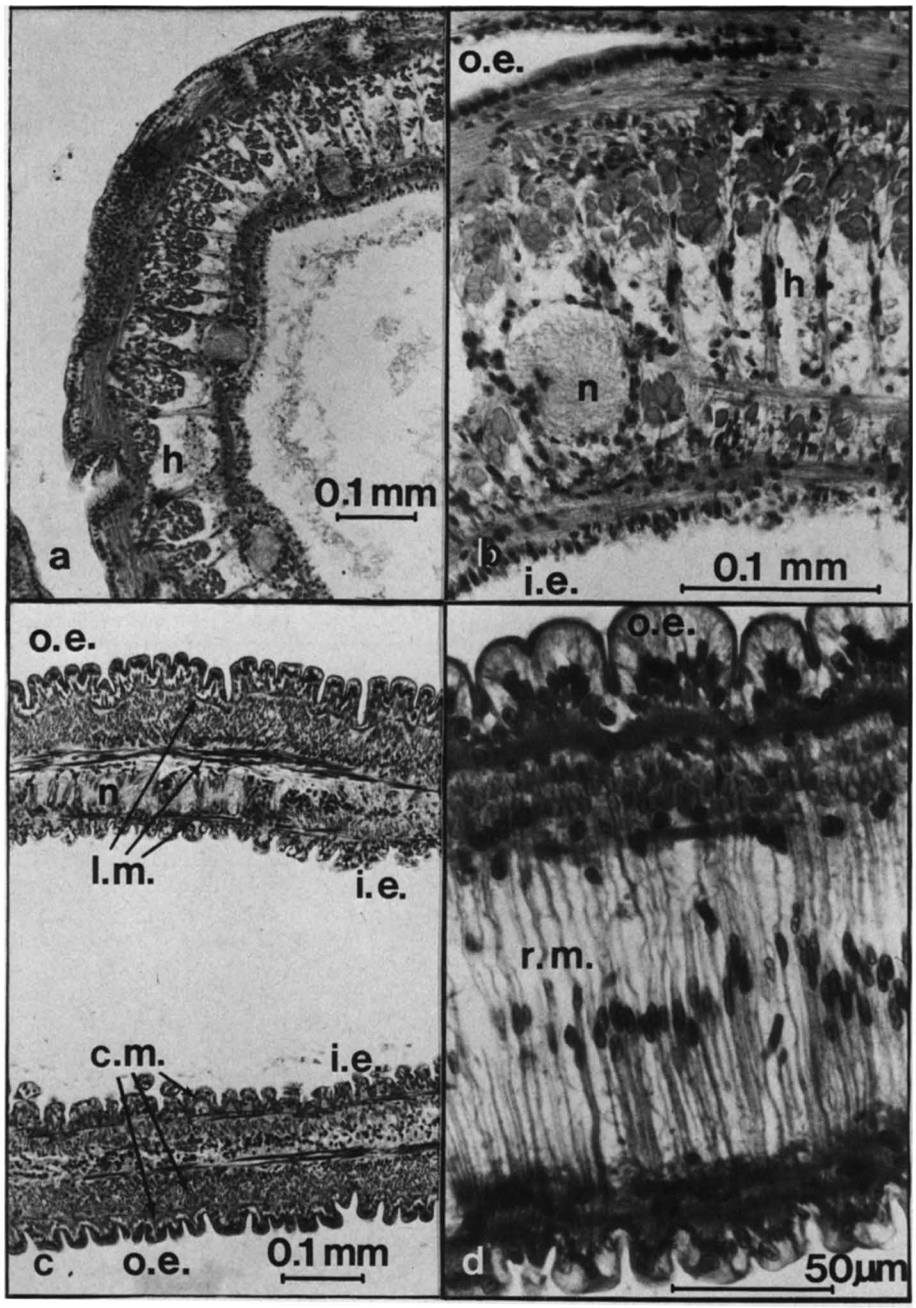

Fig. 3. Siphons of unexposed specimens of *M. balthica*. a) Cross section of an exhalant siphon. b) The same siphon at higher magnification. c) Longitudinal section of an exhalant siphon. d) Longitudinal section of the wall of an inhalant siphon. Stainings in a) and b) Masson — Gomori, in c) and d) Crossmon's. Explanations: h = lumen of haemocoele, n = nerve, i.e. = inner epithelium, o.e. = outer epithelium, c.m. = circular muscle fibres, l.m. = longitudinal muscle fibres, r.m. = radial muscle fibres.

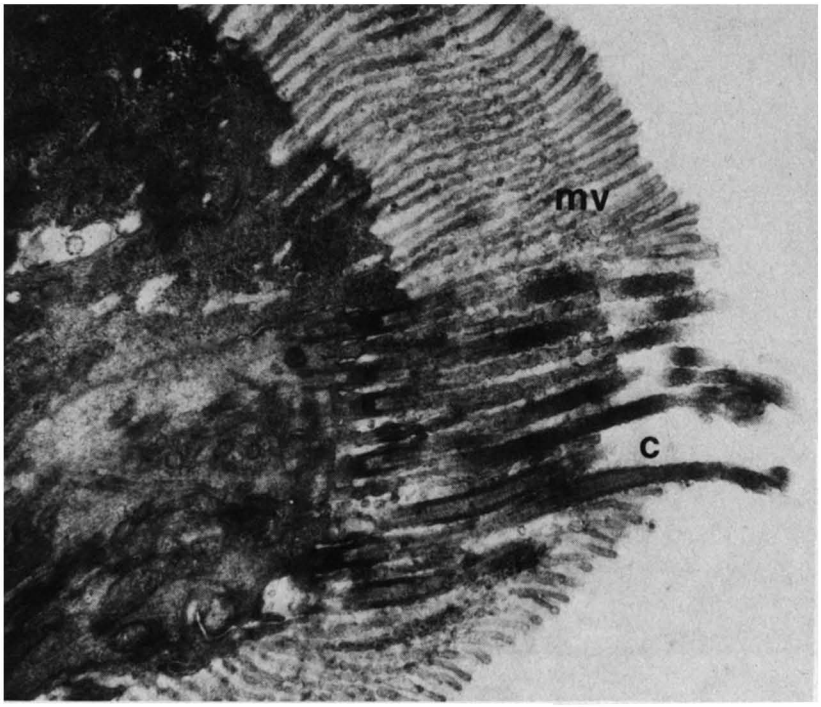

Fig. 4. Electron micrograph of the outer epithelium of a siphon. The free end of the epithelial cells bears microvilli (mv). One cell which has also a tuft of cilia (c = cilium) is also seen in the epithelium. Magnification about 17 000 x.

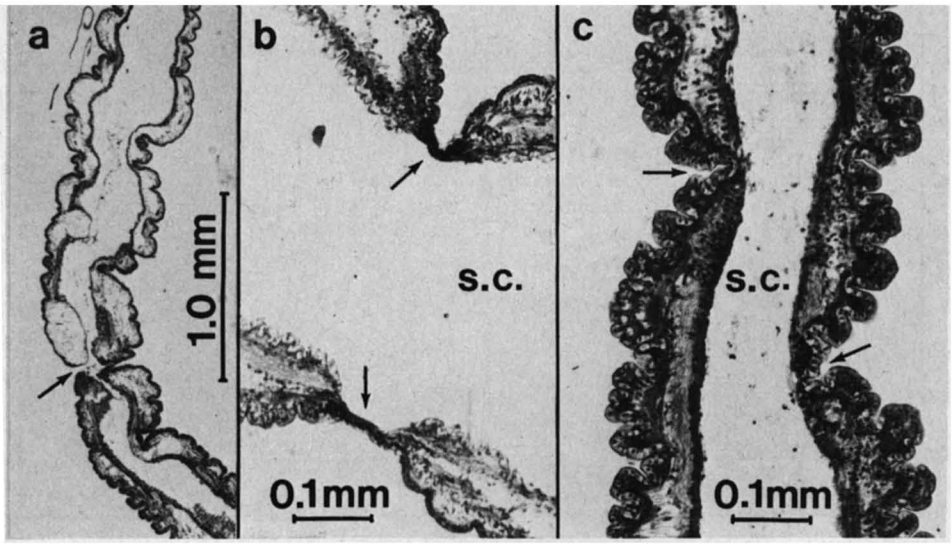

Fig. 5. Histological sections of siphons of *M. balthica* after exposure to heavy metals: a) The siphon of an animal exposed to 5 ppm Cd for 24 h. The distal end points upward. The walls show constrictions and swellings and the siphon is about to break off at the point indicated by the arrow. b) Constrictions (arrows) in the broken-off piece of siphon of an animal exposed to 20 ppm Zn for 48 h. c) A broken-off piece of siphon of an animal exposed to 0.05 ppm Hg for 24 h. The siphon is constricted (arrows) and has lost the inner epithelium. s.c. = cavity of siphon. Stainings: a) Alcian blue — PAS — Mayer's haematoxylin, b) and c) Crossmon's staining.

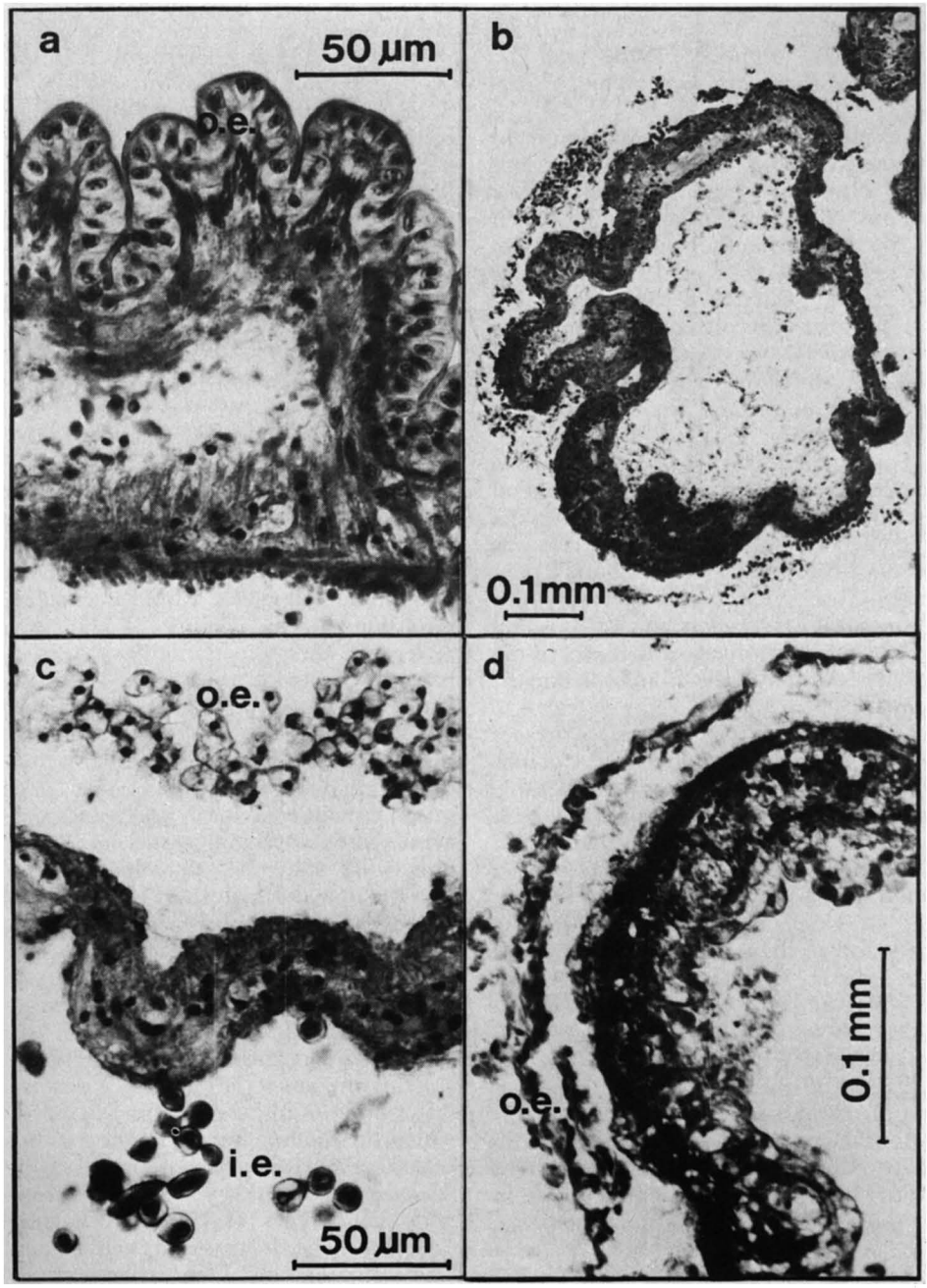

Fig. 6. Broken-off pieces of siphons of M. *balthica* after exposure to heavy metals for 24 h. a) 0.2 ppm Hg, longitudinal section: The inner epithelium is lost and the internal layers of the wall are necrotic. b) 0.5 ppm Hg, cross section: The siphon is wrinkled, both epithelia are loosened, and the muscular tissue is badly damaged. c) 0.5 ppm Cu, longitudinal section of the wall: Note the loosened vacuolized epithelia and the pyknotic nuclei. d) 1.0 ppm Cu, cross section. Stainings: a) Masson — Gomori, b) Masson — Goldner, c) Alcian blue — PAS — Mayer's haematoxylin, d) Crossmon's staining.

activity of the larvae of the fiddler crab *Uca pugilator* (DeCoursey & Vernberg 1972) and to arrest the growth rate of the clonal hydroid *Campanula flexuosa* (Stebbing 1976) and the bivalve larvae of *Crassostrea virginica* and *Mercenaria mercenaria* (Calabrese et al. 1977).

Low concentrations of Cd and Zn stimulated burrowing activity, but higher concentrations inhibited it. A similar stimulating effect of low concentrations of Cd on organisms has been observed by other authors (MacInnes & Thurberg 1973, Berland et al. 1977, Thurberg et al. 1977, Reish & Carr 1978). Stebbing (1976) has listed a number of records of the stimulating effect of heavy metals on organisms, mainly on their respiration and growth rates. The metals listed were Hg, Cu, Zn and Ni. Reish & Carr (1978) found that Pb, too, had a stimulating effect. In our experiments *M. balthica*, when exposed to Cd, seemed more sensitive to this metal in August than in January and February: the stimulating effect of 0.5 ppm concentrations was more evident, the inhibiting effect of 1 and 2 ppm concentrations was greater and mortality was higher (Eldon & Kristoffersson 1978). This difference was probably due to differences in the physiological condition of the animals in August and in January.

Cu and Zn are known to be necessary in traces for the enzyme activity of molluscs (cf. Coombs 1974). Molluscs accumulate considerable amounts of Cu and Zn in their tissues (Brooks & Rumsby 1965). Sublethal concentrations of Cu and/or Zn have been shown to depress oxygen consumption in the mud snail *Nassarius obsoletus* (MacInnes & Thurberg 1973), to reduce the growth rate and feeding intensity in the plaice *Pleuronectes platessa* (Blaxter 1977), to affect osmoregulatory function in the crabs *Carcinus maenas* and *Cancer irroratus* (Thurberg et al. 1973) and to retard settling and behavioural development in larvae of the oyster *Crassostrea gigas* (Boyden et al. 1975).

Because of the poor solubility of lead nitrate in sea water (cf. Schulz-Baldes 1972), the results of the tests with Pb are more difficult to interpret. In spite of salt complex formation, however, burrowing activity was clearly reduced at concentrations of 0.5 ppm and higher. Pb has been shown to depress the growth of the brine shrimp *Artemia salina* (Brown & Ashanullah 1971) and to retard the embryonic development of the mussel *Mytilus galloprovincialis* (Hrs-Brenko et al. 1977).

With Ni, as with Cd, the 'p.i.e.' curve is narrow and the recovery of exposed animals was poor, although none died. Except at very high concentrations Co was the least poisonous of the metals tested, and recovery was good.

Whatever the mechanism of 'poisoning' may be, the surface attacked plays a key role. In *Macoma* the epithelial surface on the siphons and on the foot, which bears microvilli (Fig. 4) and not a cuticle, can hardly serve as a barrier against dissolved substances, even if these cause no damage. The broken-off siphons seen in these tests with heavy metals coincided with histopathological changes in structure seen in light microscopy. Maybe at EM level changes would have been detectable even earlier and at lower concentrations. The fact that more than 30 % of the animals never lost parts of their siphons and that the severity of the damage varied individually may be due not only to differences in resistance between individuals, but also to differences in the action of the siphons, i.e., whether these were stretched or contracted during exposure. With Zn the siphons were often strongly contracted into the shells, and so suffered only minor damage. Rapid recognition of a hazardous agent, closure of the valves and anaerobic energy metabolism may reduce the harmful effects for some time. The capacity for anaerobic energy metabolism, the tightness and impermeability of the valves, and the ability to tolerate, detoxicate or even take advantage of small amounts of imbibed metal ions penetrating into the mantle cavity in anaerobiosis may vary greatly from species to species. Inhibition of burrowing may be an adaptation enabling *Macoma* to avoid high short-term concentrations of heavy metals (for burrowing exposes large areas of surface epithelium). But in these experiments it must rather be considered an indication of inability to burrow (cf. tests and recovery tests in clean aquaria). The stimulation of burrowing seen at low concentrations of Cd and Zn would be a useful response if the chemical is dissolved in the water and not dispersing from the sediment into which the mollusc burrows. The function of the occasional ciliated cells found in the epithelium of *Macoma* siphons is as yet unknown. In cephalopods some types of ciliated cells have been suggested to be chemosensory cells (Emery 1975). The microanatomy, innervation and function of the siphons of *Macoma* are under investigation.

Several authors have reported histological changes in various organisms caused by heavy metals. Nimmo et al. (1977) found blackened and necrotized gill tissue in the shrimps *Penaeus duorarum* and *Palaemonetes vulgaris* after exposure to Cd. Gardner & Yevich (1970) found pathological

changes in the intestinal tract, kidney, respiratory lamellae, and eosinophils and thrombocytes of the killifish *Fundulus heteroclitus*, caused by Cd poisoning. These examples give some idea of the broad spectrum of poisonous effects of Cd. To take two further examples, Zn causes morphological variations in the dinoflagellate *Scrippsiella faeroense* (Kayser 1977), and Ni causes morphological changes to the larvae of the bivalve *Mercenaria mercenaria* (Calabrese et al. 1977). Thus most heavy metals may cause morphological and behavioural changes in organisms. The nature of these changes depends on the relationship between the organism and the metal.

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