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UPTAKE AND RELEASE OF MERCURY AND CADMIUM IN VARIOUS ORGANS OF MUSSELS (*ANODONTA CYGNEA* L.)

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Accumulation of heavy metals in aquatic organisms - similarly to other living systems - is the result of two opposite processes, namely the net result of uptake and release. In case uptake is faster than release, accumulation takes place, in case the two processes are equal, there is an equilibrium.

The mechanisms which are responsible for the uptake and release may function differently depending on the chemical character of the substance taken up by the organism, and often also the environmental concentration of the substance is of primary importance. At low concentrations equilibration usually takes place at a low level of accumulation, while higher concentrations may lead to high accumulation. The best biomonitors for detection of pollution are the organisms with a great accumulation capacity, the regulatory mechanism of which are week to keep the equilibrium of uptake and release at low level even at low environmental contamination.

It is known that mussels are good accumulators for a number of heavy metals (Pringle et al. 1968). Since they are characterized by a filter feeding behvior, when they pump the water through the gills and take up large amounts of small particles as food, they ingest various substances dissolved in the water or incorporated into algae, bacteria and other particulate substances.

In our earlier investigations we showed that the fresh water mussel Anodonta cygnea L., living in Lake Balaton, similar to the other molluscan species, accumulates high amounts of

heavy metals, and its concentration capacity may reach a value of several ten thousands in various organs as compared to the concentration of the metal in the surrounding water (Salánki et al. 1982). The concentration capacity of various organs is different, the highest amount of accumulated metals was detected in the kidney and gills, while the lowest levels were detected in the adductors (V.-Balogh and Salánki 1984).

The great concentration capacity of mussels to heavy metals allows us to use these animals in biomonitoring the heavy metal pollution of the environment which may occur as a result of various kinds of human activities. The question arises however, whether temporary changes of heavy metal concentrations in the environment will be reflected in the heavy metal content of mussels or not, and, on the other hand, whether changes in the heavy metal content of mussels reflected changes in the environment or it may be connected with mechanisms within the animal regulating uptake and release of these substances.

In the literature there are contradictions whether heavy metals will be released by mussels, or not, in case the surrounding metal concentration drops. Mason et al. (1976) did not found release of Hg from oysters within 256 hours. The same was reported for Pb and Cd by Klumpp and Burden-Jones (1982) in Trichomya hirsuta. On the other hand, Cunningham and Tripp (1973) found a considerable release of accumulated Hg by oysters kept in Hg-free water. Fowler et al. (1978) described also release of Hg by Mytilus, however, to a much lesser degree. They also found a redistribution of the metal within the animal during the depuration period. Looking for the release of Cd Zaroogian (1979) did not find real depuration within 16 weeks from oysters having been previously kept in a 15 µg/l solution of cadmium.

In a series of investigations we translocated mussels (Unio sp.) of a low metal content from Lake Balaton into the River Zala which is the main inflow into the lake. Starting at early spring, we took samples from these mussels at two-week intervals for measuring heavy metal concentration in the gills (V.-Balogh and Salánki 1983). For Hg, Cd, Pb and Zn continual increase was found, however, on some occasions, also a considerable drop in

metal concentrations. These results suggest that the metals taken up by mussels are released if the surrounding medium changes, and so mussels can be used as biomonitors for detecting fluctuation of heavy metal pollution. Nevertheless, it seems necessary to carry out laboratory experiments and to clarify the dynamics of uptake and release of toxic metals in vaious organs of the mussels. We report here our results concerning uptake and release of Hg and Cd in the tissues of Anodonta cygnea L.

MATERIAL AND METHODS

In the experiments 11.9±1.01 cm long specimens of Anodonta cygnea were used taken from fish ponds. The animals had been kept in running Balaton water for weeks before the heavy metals were added. No additional feeding was performed. Two separate series of experiments were carried out both with Hg and Cd. First we investigated the uptake of metals both at short intervals during 24 hours and at long intervals during up to 840 hours. In the second series after an 840 hours' uptake we studied depuration, in case of Hg for 840, in case of Cd for 672 hours. Hg was applied in the form of $HgCl_2$, while Cd as $CdSO_4$. The following system during the uptake experiments ensured a constant metal concentration which was controlled in the outflow from time to time.

When investigating only uptake the animals were kept separately in 3 l volume Plexyglass chambers. This amount of water was renewed within about one hour due to the perfusion system. The concentration of Hg and Cd were 10±7 and 16±5 µg/l, respectively. Temperature of the water changed between 7-15 °C.

In the second series of experiments when long-term uptake and release were investigated, 100 animals were placed together in a large wessel containing 100 l water. The in- and outflow assured the total change of the water within 5 hours. The Hg and Cd concentrations were 12.9±2.8 and 6±1.3 µg/l, respectively. The water temperature varied between 15-22 °C.

For measuring heavy metal concentrations in each case 3 animals were used parallelly. Gills, adductor muscles, mantle,

kidney and foot were sampled separately. In case of measuring Hg the whole foot (including viscera) was used, while in Cd measurements only of the viscera located in the foot were analysed.

Tissue samples for Hg were prepared according to Paus (1972), and for Cd measurements according to Krishnamurty et al. (1976) Measurements were carried out by atomic absorption spectrophotometry as it was published earlier (Salánki et al. 1982)

The concentration factor was calculated according to Taylor (1983), $CF=\frac{Ce-Cc}{Cs}$, where Ce=metal concentration in the organ at the end of the exposure, Cc=metal concentration in the organ of the control animal, Cs=metal concentration of the water during the experiment.

RESULTS AND DISCUSSION

Concentrations of Hg and Cd in the organs of Anodonta cygnea L. before exposure to heavy metals are given in Table 1.

Table 1: Concentration of Hg and Cd in control animals (µg/g dry w±SEM)

Organ	Hg		Cd	
	1st series	2nd series	1st series	2nd series
gills	1.21±0.931	0.669±	3.49±0.383	4.24±0.824
foot	1.19±0.786	0.201±0.123	--	--
adductors	1.33±0.806	0.868±0.577	3.67±1.03	4.94±1.29
mantle	1.25±0.088	0.397±0.155	2.67±0.556	3.89±1.88
kidney	1.74±0.692	1.051±	11.6 ±0.961	10.3 ±1.00
viscera	--	--	4.45±0.416	6.36±0.670

The values show that there were some differences between the control concentrations in the two series of experiments, especially for mercury. Nevertheless, due to the large uptake of metals during exposure, we do not ascribe importance to these differences when evaluating the dynamism of uptake and release.

UPTAKE AT THE BEGINNING OF EXPOSURE

Following exposure of mussels to 10 µg/l Hg solution the concentration of Hg increased in all organs within 30-60 min, however, in a few hours it dropped everywhere below the control (Fig.1). Following further exposure an increase started again first in the gills and later in all organs, reaching a moderate elevation by 24 hours. The concentration of Cd increased during the first 24 hours following the exposure of mussels to 16 µg/l Cd only in the kidney, which was noticeable already within 1 hour (Fig.2), but it also decreased somewhat between 12 and 24 hours.

UPTAKE OF Hg AND Cd DURING 840 HOURS' EXPOSURE

Taking samples after 72, 168, 336, 504, 672 and 840 hours' exposure we found that the uptake of both Hg and Cd increased linearly in most organs during this period (Table 2). Our results are in agreement with the findings of Mason et al. (1976) who described in oysters a two-phase uptake, the first phase being logarithmic, while the second one linear. Nevertheless, in gills a saturation with Hg and in the kidney and viscera with Cd were found, since after 672 hours of exposure to 10, and 16 µg/l mercury and cadmium concentrations, respectively, no further increase of the metal concentration was observed.

At the end of the exposure period the concentration factor (CF) was rather different for different organs (Table 3). Accumulation of mercury was of a higher degree than that of the cadmium, and there were also differences between the two metals according to the affinity of organs. The kidney revealed very high CF for mercury, while the viscera and mantle showed prominent values for Cd. The lowest accumulation was observed for both metals in the adductor muscles.

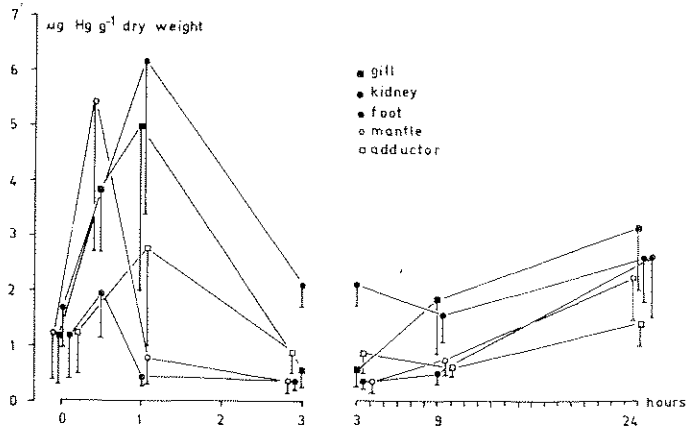

Fig.1. Uptake of mercury into various organs of Anodonta cygnea L. exposed to 10 µg/l solution of Hg during the first 24 hours

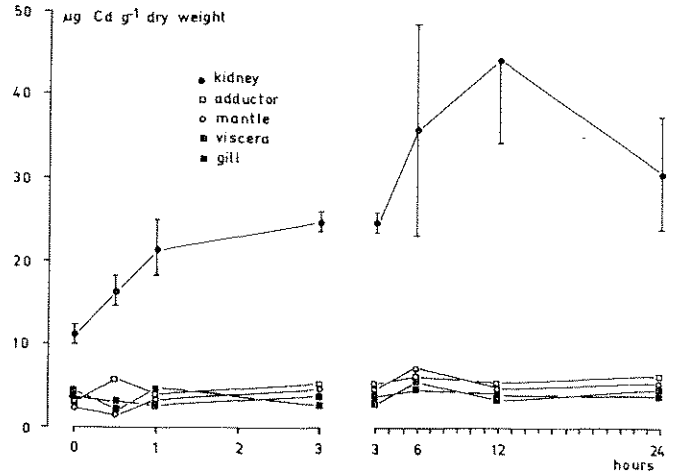

Fig. 2. Uptake of cadmium into various organs of Anodonta cygnea L. exposed to 16 µg/l Cd during the first 24 hours

Table 2. Linear uptake of Hg and Cd in organs of Anodonta cygnea L. during 72-840 hours' exposure

Organ	Hg /10 µg l^{-1}/	Cd /16 µg l^{-1}/
gills	⊗	y = 3.61 + 0.028 x r = 0.782
foot	y = -2.79 + 0.074 x r = 0.851	-
adductors	y = 0.462 + 0.031 x r = 0.846	y = 6.43 + 0.010 x r = 0.593
mantle	y = -6.46 + 0.136 x r = 0.883	y = 2.49 + 0.057 x r = 0.945
kidney	y = -28.6 + 0.593 x r = 0.826	⊗
viscera	-	⊗

⊗ Saturated before 840 hours
y = equation of the line
r = regression coefficient

Table 3. Concentration factor (CF) in various organs of Anodonta cygnea L. after 840 hours' exposure

Organ	Hg /10 µg l^{-1}/	Cd /16 µg l^{-1}
gills	8000	2000
foot	7000	-
adductor muscle	3000	500
mantle	10000	3000
kidney	50000	2000
viscera	-	3000

DEPURATION OF Hg AND Cd

In this second series of experiments with mercury lasting altogether for 1680 hours and with cadmium for 1512 hours during the uptake period we measured the metal concentrations of the organs three times, namely at 168, 504 and 840 hours of the exposure. Following 840 hours' exposure, the animals were washed in running, metal-free Balaton water. Samples were taken at

72, 168, 336, 504, 672 and 840 hours to check mercury depuration, while for measuring cadmium depuration the last sample was taken at 672 hours. We could not sample mussels treated with cadmium at 840 hours of depuration, because the mortality of cadmium-treated animals became very high after placing them into metal-free water.

Elimination of mercury

Mercury, taken up within 840 hours was not released in equal degrees from various organs, and there was no total depuration from either of them during the experimental period. Depuration of mercury was fastest from the kidney (Fig.3), the adductor muscle (Fig.4) and the mantle (Fig.5), where decrease of the accumulated metal to 50 per cent was observed between 72 and 168 hours ($T_{1/2}$=half depuration time). Nevertheless, even after 840 hours these organs contained 35, 7 and 54 times more

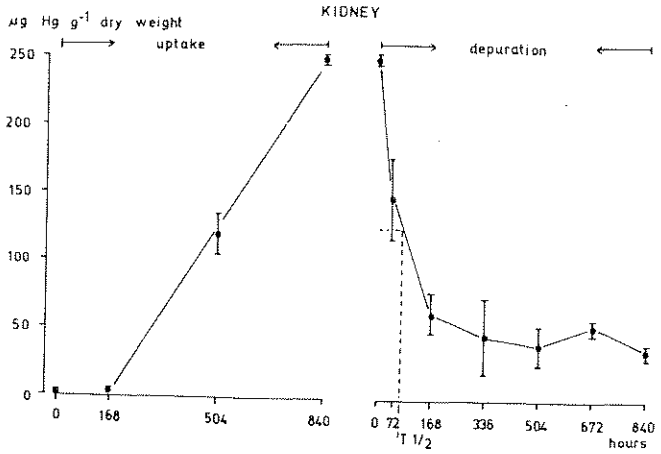

Fig.3. Changes of concentrations of Hg in the kidney of Anodonta cygnea L. during uptake and depuration experiments. $T_{1/2}$=time, necessary for the 50 per cent decrease of the metal concentration.

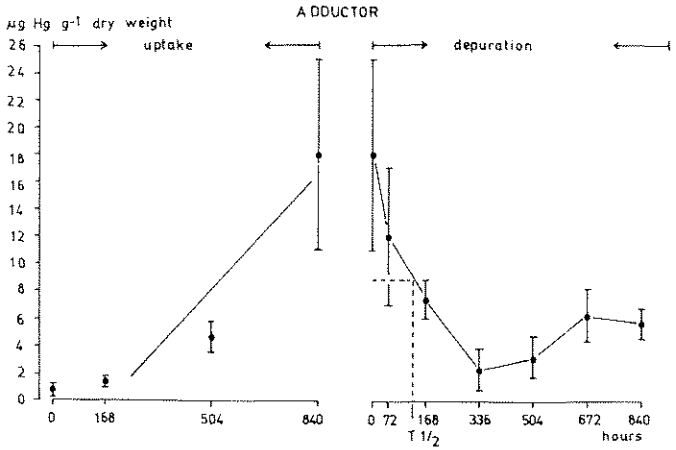

Fig.4. Changes of concentrations of Hg in the adductor muscle of Anodonta cygnea L. during uptake and depuration experiments

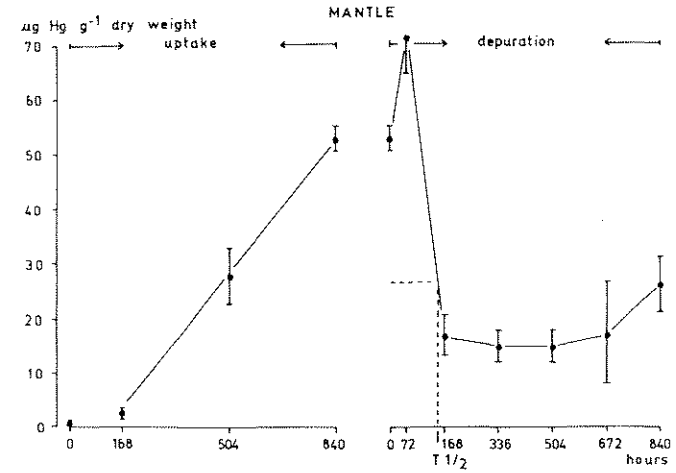

Fig.5. Changes of concentrations of Hg in the mantle of Anodonta cygnea L. during uptake and depuration experiments

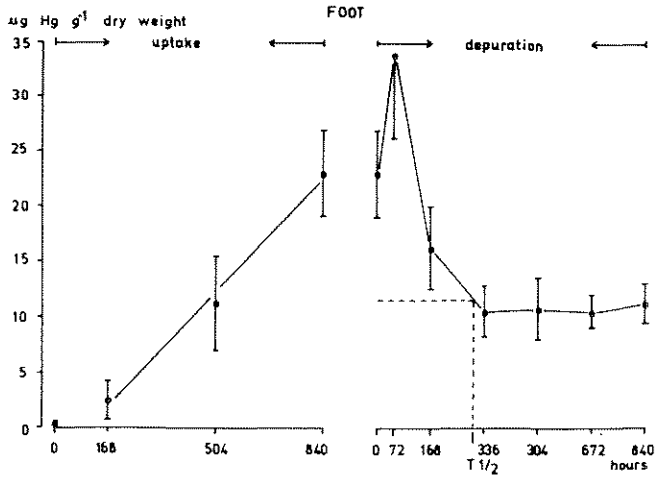

Fig.6. Changes of concentrations of Hg in the foot of Anodonta cygnea L. during uptake and depuration experiments

mercury than the control. $T_{1/2}$ for the foot (Fig.6) was between 168-336 hours, and this level, exceeding the control 54 times, remained constant to the end of the experiment. Less depuration was observed in gills (Fig.7), where $T_{1/2}$ had not been reached during 840 hours, although a definite depuration occurred.

Elimination of cadmium

Binding of cadmium was in each organ stronger than that of mercury. $T_{1/2}$ was between 504 and 672 hours for the mantle (Fig.8) and the gills (Fig.9). There was an obvious depuration from the adductors (Fig.10), however, no half depuration was observed up to 672 hours. In case of the viscera (Fig.11), a 20-25 per cent decrease of cadmium concentration was measured within one week, however, further release was not observed. The kidney (Fig.12) did not release cadmium within 672 hours, there was even a transient increase of cadmium concentration.

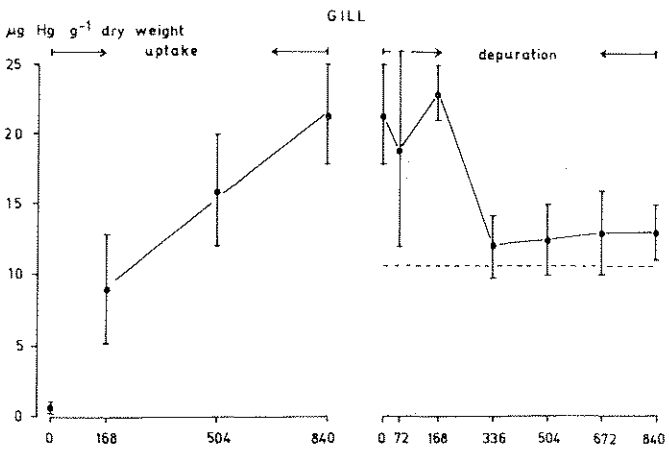

Fig.7 Changes of concentrations of Hg in the gills of Anodonta cygnea L. during uptake and depuration experiments

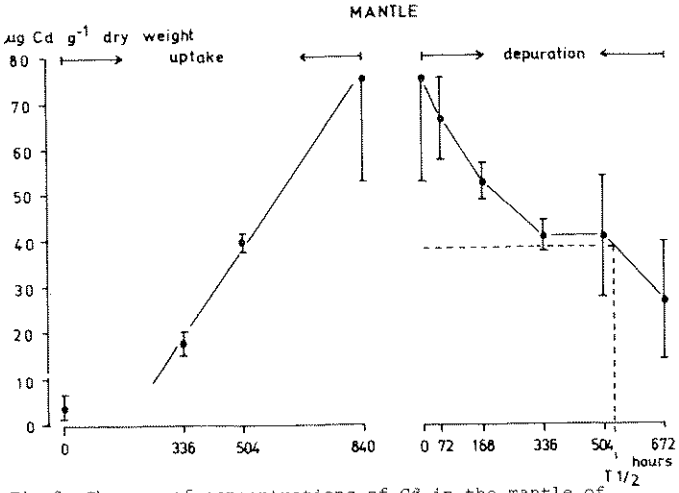

Fig.8 Changes of concentrations of Cd in the mantle of Anodonta cygnea L. during uptake and depuration experiments

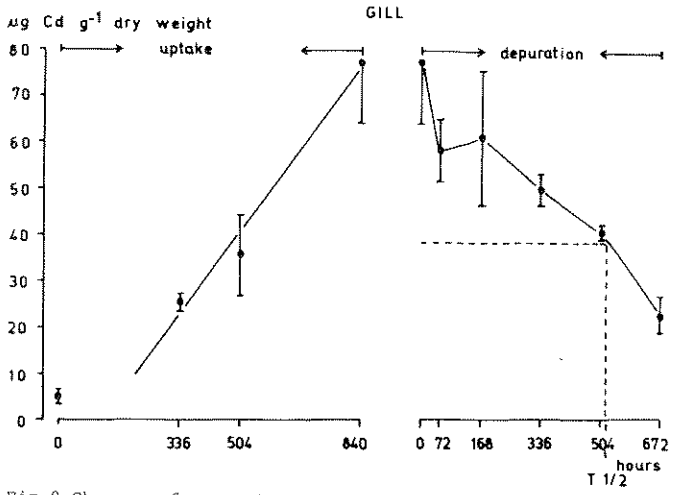

Fig.9 Changes of concentrations of Cd in the gills of Anodonta cygnea L. during uptake and depuration experiments

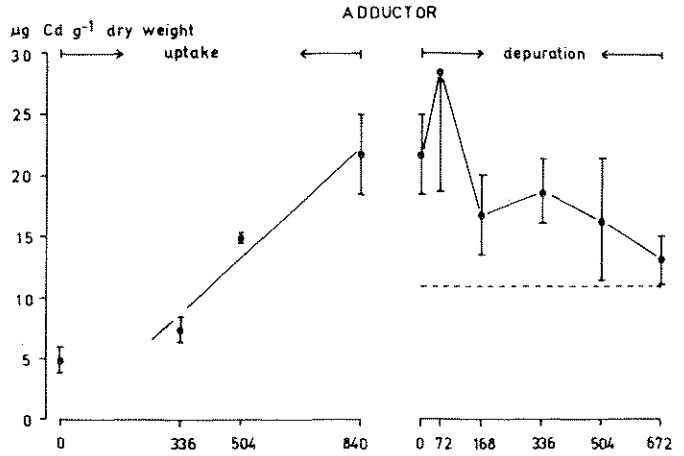

Fig.10 Changes concentrations of Cd in the adductor muscles of Anodonta cygnea L. during uptake and depuration experiments

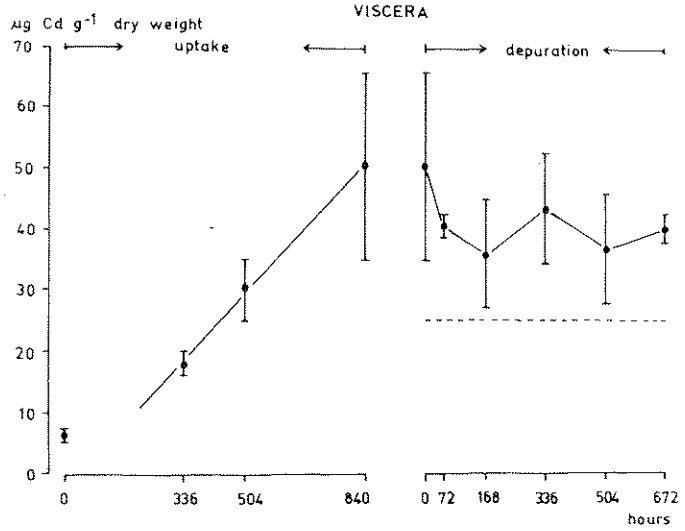

Fig.11 Changes of concentrations of Cd in the viscera of Anodonta cygnea L. during uptake and depuration experiments

Our results are in agreement with the data of Ruzic (1972) Mason et al. (1976), George and Coombs (1977) and others, showing that heavy metal accumulation has two phases in mussels, the first being a reversible one, while the other an irreversible process. In our experiments we found that the various organs accumulate heavy metals to a different degree, the order for mercury being: kidney > gill > mantle > foot (viscera) > adductor muscle, while for cadmium: kidney > viscera > mantle > gill > adductor muscle. The prominent role of the kidney in metal uptake was emphasized also by Bryan (1973) and Carmichael et al. (1980). It is noteworthy that, among the organs, the gill was a better accumulator for mercury than for cadmium. This refers to a difference in the mechanism of uptake and storage of Hg and Cd by the gills, although both metals are known to bind to the SH groups of proteins (Vallee and Ulmer 1972).

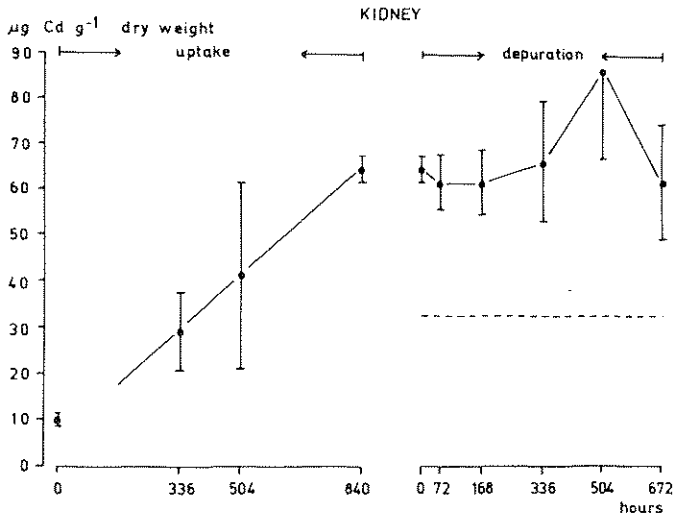

Fig.12 Changes of concentrations of Cd in the kidney of Anodonta cygnea L. during uptake and depuration experiments

Not only uptake but also depuration of Hg and Cd were different from various organs of mussels. In general, release of Hg was faster than that of Cd. The order of organs for the release of Hg was: kidney > adductor muscle > mantle > foot > gills. The gills kept more than half of the Hg after 840 hours' depuration time. Although in similar conditions the accumulation of Cd was less than that of Hg, the release was slower, and the order of organs was mantle > gills > adductor muscle > viscera and kidney. The viscera and kidney did not practically release Cd within 672 hours, suggesting a strong binding. The high mortality of Cd-treated mussels during this depuration period can, possibly be connected with this phenomenon.

Our results support these findings, showing that there is a release of Hg and Cd from mussels (Cunningham and Tripp 1973, Fowler et al. 1978). However, this is not the same concerning

the two metals and is different especially for the gills, kidney and viscera.

The results offer a practical guideline for using mussels for monitoring a steady or fluctuating Hg and Cd pollution. Due to the high concentration capacity the best organs for monitoring a steady mercury pollution of the water are the kidney and gill, while in case of Cd pollution kidney and viscera can be recommended. In case the concentration of heavy metals fluctuates in the water, the drop of mercury can be monitored in the kidney, while that of cadmium in the mantle and gills. Due to the low rate of depuration in metal-free water, the gills will reflect mercury pollution for a long time, while for cadmium the kidney can be used for this purpose. These specificities should be taken in consideration when mussels are translocated into a new environment for studying the Hg and Cd pollution in a timely manner.

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DISCUSSION

SHIBER J G: Was the experimentation all conducted in Balaton water? Was the water filtered in order to reduce any possible interference from debris, microorganisms, etc.?

SALÁNKI J: The Balaton water we use in the laboratories is not filtered, only sedimentation of mud takes place in the containers which are at the top of the Institute, where the water is pumped up directly from the Lake. Our intention was to maintain those feeding conditions for the animals.

THEEDE H: The two-phase uptake of heavy metals is an interesting phenomenon. What happens after the first hour of uptake? If there is a preliminary release, e.g. of mercury, what explanation can be given to this? Does this two-phase phenomenon also occur at very low levels of contamination?

SALÁNKI J: The fast uptake and release during the first hours of exposure refers to an active mechanism in the animal which tries to eliminate heavy metals. Certainly at the beginning of the exposure the heavy metals are taken up but are not bound in the tissues, therefore they are eliminated. The slow uptake mechanism may also function already at the first hours, but the stored metal is still very low. Later this second phase predominates, and the metals taken up by this mechanism cannot be easily eliminated.

THEEDE H: Can you give some additional information about the experimental conditions and the food supply during the longterm courses of activity registrations of mussels? Availability of food will be a very decisive factor for the activity pattern.

SALÁNKI J: We did not measure the food content of the water and did not complement the food available in the Balaton water. Algae, microbes and some part of detritus were present but those were absent which were too heavy to sediment in the mud.

THEEDE H: During the depuration phase a release of mercury from different organs was described; only the kidney continued to accumulate. What happens with the hepatopancreas?

In our experiments with Mytilus edulis it could be shown that a redistribution of cadmium took place after the animals had been transferred into clean sea water subsequent to prior accumulation. Some organs lost part of the cadmium, whereas

kidney and hepatopancreas continued to accumulate. Altogether the whole body burden nearly did not change during the course of some weeks in the case the animals had accumulated this metal from low Cd-concentrations in sea water.

SALÁNKI J: We did not investigate the hepatopancreas separately, but together with the viscera (gut, genital organs, etc.) Your findings on the exhcange of cadmium between kidney and hepatopancreas during depuration experiments are very interesting, and really it could give an explanation for a part of our results. Thank you for your comment, - we shall make experiments to check this possibility.

WACHS B: You will take Anodonta as indicator for Hg and Cd pollution. Why do you choose such very high concentrations in your experiments? Are we sure that the accumulation behaviour of mussels will be the same or similar to these results under field conditions in polluted or strongly contaminated rivers?

SALÁNKI J: The concentrations we used in laboratory experiments is naturally higher than in lakes and rivers, but in some places similar concentrations may appear (e.g. industrial waste waters). We suppose, that both the uptake and release of metals, as well as the reaction of bivalves are similar to that we observed in the laboratory. We intend to conduct experiments with 10-100 times lower concentrations as well, in order to get closer to field situations.

LORCH D: I was gratified to see in your slide that mussels react similarly to algae when mercury is accumulated. With algae we too found first a fast metal (lead) accumulation and after about 1 to 2 h a reduction in metal concentration followed by a long term lead accumulation.

How does the total cadmium content of the animal behave during depuration?

SALÁNKI J: We did not measure the total Cd content, since the animals were rather large (wet weight of one specimen is about 100-120 g), but one could calculate it on the basis of the weight of the organs. Since there was a reduction in all organs during depuration (except the kidney), there was obviously release of Cd from the soft body.

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INTERACTIONS OF HEAVY METALS AT TISSUE AND CELLULAR LEVEL IN AQUATIC ORGANISMS

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There is much current concern about the increasing concentrations of heavy metals in both inland and coastal waters resulting from increased industrial activity such as mining (e.g. see Bryan, 1979). Acid rain has also been shown to be responsible for the increase in heavy metal concentrations in a number of freshwater environments (e.g. see Henricksen and Wright, 1978; Wittman, 1981). This is mainly due to metal-containing air-borne particulate matter in the form of rain or snowfall. Also of importance is the resultant increased solubility of bound metal in acid lakes in comparison to neutral waters in the adjoining areas.

As a result of concern towards the aquatic environment, much interest has been shown in the biological and toxicological effects of heavy metals in a number of aquatic organisms. Numerous studies with heavy metals (Fig. 1) have demonstrated their toxic effects (Martin et al., 1981); bioaccumulation (Bryan, 1979; Simkiss et al., 1982); excretion (Simkiss et al., 1982) and binding to e.g. metallothioneins (Roesijadi, 1980). However, little information is available in the literature concerning the mechanism of action of either lethal or sublethal levels of heavy metals before being immobilized or excreted by the organism (Fig. 1). This contribution delineates three physiological effects brought about by copper, but not by zinc, which depend both on the nature of the cell as well as its particular physiological status:

(1) SIPHONAL TISSUE CONTRACTION IN THE MARINE BIVALVE MOLLUSC SCROBICULARIA PLANA

The use of estuarine bivalve molluscs, e.g. Scrobicularia plana and Mytilus edulis, as possible indicator organisms for environmental pollution has been suggested (e.g. see Phillips, 1977; Bryan, 1979). The first visible response occurring when marine bivalve molluscs are exposed to lethal levels of heavy metals is withdrawal of the siphons which is then followed by valve closure (Akberali and Black, 1980; Akberali et al., 1981). Application of both copper and zinc to in situ siphonal tissue of Scrobicularia leads to siphon contraction (Akberali et al., 1981). However, application of comparable zinc concentrations has no apparent effect on the isolated siphonal tissue (Fig. 2). Removal of zinc followed by application of copper results in contraction/relaxation of the isolated siphon with the siphon remaining in a contracted state. These results indicate that copper and zinc have different modes of action on the isolated siphonal tissue