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THE DYNAMICS OF MERCURY AND CADMIUM UPTAKE INTO DIFFERENT ORGANS OF *ANODONTA CYGNEA* L.

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Abstract—The bioaccumulation of Hg and Cd into various organs of the freshwater mussel *Anodonta cygnea* was investigated during an 840 h experimental period. Parallel with the metal concentrations the periodicity of activity and the body weight of the animals were also checked.

The accumulation of Hg in all of the investigated organs, and that of the Cd into the kidney have two phases, up to 24 h it was non linear, between 24 and 72 h it became linear in most organs up to 840 h, and could be characterized by a regression line. For Hg accumulation the gills while for Cd accumulation the kidney and viscera were exceptions, here saturation was observed after 504 and 672 h, respectively.

The speed of Hg and Cd uptake was different in various organs of *Anodonta cygnea*. Into the kidney the accumulation of Hg was six times faster than that of Cd. As compared to the adductor muscles the Hg and Cd uptake of the kidney was 20 and 10 times faster, respectively.

The factor of concentration was in all organs except adductor muscles over 1000, in case of the kidney the rate of bioconcentration for Hg reached nearly 100,000.

The dry weight of organs dropped during the metal uptake by 20–60%, but no mortality was observed up to 840 h.

In the presence of Hg and Cd the filtering activity of the mussels became reduced as a result of increase of inactive rest periods. Nevertheless, the decrease of activity did not prevent the linear uptake of metals into the animal

Key words—heavy metals, mercury, cadmium, bioconcentration, freshwater mussel, *Anodonta cygnea*, filtering activity, concentration factor, gills, kidney, adductor muscles

INTRODUCTION

It is well known that among water animals, mussels are good accumulators of heavy metals (Butler *et al.*, 1971; Roberts, 1976), therefore they are suitable test organisms for indicating low level metal pollution of natural waters (Majori and Petronio, 1973; Goldberg *et al.*, 1978). At the same time some heavy metals are known to reduce filtration activity of mussels (Salánki, 1966, 1977) and in this way they inhibit both feeding and growth of these animals. Accumulation of heavy metals is different in various organs. The highest concentration of metals was found in the gills of both marine (Zaroogian, 1980) and fresh water species (Salánki *et al.*, 1982) collected from their natural habitat. In mussels taken from Lake Balaton we found that the concentration of seven heavy metals (Hg, Cd, Cu, Pb, Zn, Fe and Mn) was 1000–10,000 times higher in the gill then in the water, while in other organs (mantle, adductor muscles and foot) the rate of accumulation was usually 10 times lower (Salánki *et al.*, 1982).

When investigating, under laboratory conditions, the uptake of Cd (Zaroogian, 1980) and Hg (Mason et al., 1976) in marine mussels a continuous accumulation was found during several weeks long exposure time, and in a 2 week long experiment a linear uptake of Hg was described for the fresh water *Anodonta grandis* (Smith *et al.*, 1975). According to laboratory experiments in a concentration 10^{-5} g ml^{-1} Hg and Cd change the activity rhythm of mussels very similarly (Salánki, 1960), resulting in a reduction of the active periods. Since both Hg and Cd block mainly SH groups in proteins, it can be supposed that they affect physiological processes through a similar mechanism.

In the present investigations we wanted to clarify (a) the dynamics of the uptake of these two toxic heavy metals in the fresh water mussel when the metals are present in the water in low concentration; (b) what is the degree of the differences in the accumulation capacity of various organs; and (c) whether saturation with heavy metals can be reached during a 5 week long exposure or not. During the experimental period we recorded the pumping activity of the animals and we followed the changes in weight of the organs, since the former can influence the accumulation itself, while the latter should be taken into consideration when evaluating the rate of accumulation.

MATERIALS AND METHODS

For the experiments specimens of adult *Anodonta cygnea* L. (11.9 ± 1.0 cm length) collected from fish ponds were used. Before the experiments the animals were kept in an

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aquarium supplied with Balaton-water for 2 months or more, for acclimation. There was enough food in the water before or during the experiment that no additional food was provided.

Experiments were carried out during the winter months. The animals were placed separately in plexiglass tanks (3 l. each) with running water. The position and movement of the shells was recorded on a mussel actograph (Salánki and Balla, 1964). In parallel experiments 10 animals were used.

In the experiments Hg^{2+} was added as $HgCl_2$, Cd^{2+} as $CdSO_4$. Stock solutions were prepared and added to the experimental tank after dilution with the inflow water to the required concentration. The rate of the flow was 48 ml min^{-1} assuring the exchange of the water within 1 h and keeping the metal concentration constant around the animal. The Hg and Cd concentrations were measured in the tank from time to time.

During the experimental period average concentrations were $10 \pm 7\ \mu g\ l^{-1}$ for Hg and $16 \pm 5\ \mu g\ l^{-1}$ for Cd. The temperature of the water varied between 7–15°C in accordance with changes of the reservoir's temperature. No mortality was observed during the experiments.

The Hg concentration of the organs was measured after 0.5, 1, 3, 9, 24, 72, 168, 504 and 840 h while Cd concentration after 1, 3, 6, 12, 24, 72, 168, 336, 504, 672 and 840 h from the start of the experiments. Three mussels were analysed at each time, and another three animals served as controls before adding the heavy metals. The wet weight of tissues used for a measurement varied between 1–15 g, depending on the mass of the different organs.

Both Hg and Cd concentrations were analysed in the gills, mantle, adductor muscle and kidney. The rest of the soft parts (i.e. the foot and viscera) were used totally for Hg determination, while Cd was measured only in the viscera.

Samples were prepared for Hg and Cd determination according to the method of Paus (1972) and Krishnamurty *et al.* (1976) respectively. For the measurements an atomic absorption spectrophotometer Typ Zeiss AAS1 was used. Cd was measured in air-acetylene with flame atomization as described earlier (Salánki *et al.*, 1982), while for measuring Hg concentration an additional unit (Spectromom) was applied according to the method of Hatch and Ott (1968). Values are given to dry weight both for Hg and Cd.

The factor of bioconcentration was calculated according to Taylor (1983) on the basis of the formula $CF = C_e - C_c \cdot C_v^{-1}$, where CF = concentration factor, C_e = the metal concentration in the organs at the end of the exposure, C_c = the metal concentration in the control animals and C_v = the metal concentration of the water during the experiment.

Filtering activity of the animals was followed by recording the position and movements of the shells. Filtering takes place when the shells are open and fast pumping movements occur, while a persistent closed position of the shells marks a rest period (Salánki and Lukacsovics, 1967). We continuously recorded the shell movements, both 1 week before the experiments and during the exposure to heavy metals. The duration of the active and the rest periods are given in hours.

RESULTS

Accumulation of metals at the start of the experiment

To follow the course of the accumulation, measurements were made with shorter intervals at the beginning of the experiment. Since water enters the animal through the inhalant siphon only for open shells, at measurements less than 12 h the duration of the active periods was taken in consideration as exposure time. At measurements greater than 12 h the time given as duration of exposure includes both active and rest periods.

Table 1. Concentration of heavy metals in the organs of control animals (μg metal g^{-1} dry wt) and in water of Lake Balaton (mg metal l^{-1})

Organ	Hg $\bar{x} \pm$ SEM	Cd $\bar{x} \pm$ SEM
Gill	1.21 ± 0.913	3.49 ± 0.383
Foot and viscera	1.19 ± 0.786	—
Adductor muscle	1.33 ± 0.806	3.67 ± 1.03
Mantle	1.25 ± 0.088	2.67 ± 0.556
Kidney	1.74 ± 0.692	11.6 ± 0.961
Viscera	—	4.45 ± 0.416
Balaton water	0.00072	0.0008

$\bar{x}$ = Mean concentration; SEM = standard error of mean.

Treating the animals with the experimental solutions, the concentration of the tested heavy metals change differently in different organs (control is given in Table 1).

Exposing the animals to Hg solution, the Hg concentration increased in all organs within 0.5–1 h (Fig. 1), however, dropped below the control value after 3 h. As a result of permanent exposure the Hg concentration increased again at first in the gills, and later in other organs, but a significant uptake could be measured only after 72 h.

In the presence of Cd there was an increase of Cd during the first 24 h only in the kidney (Fig. 2). The elevation of Cd concentration was measured after

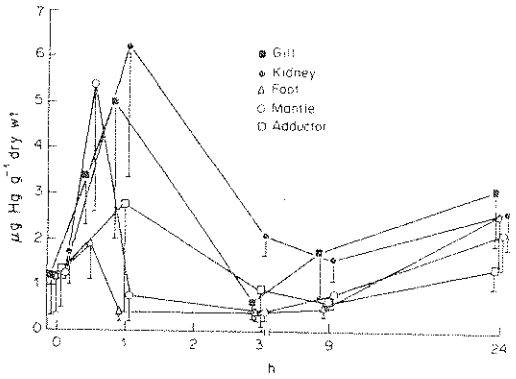

Fig. 1. Change of Hg concentration in various organs of *Anodonta cygnea* L. during 24 h exposure to 10 $\mu g\ Hg^{2+}\ l^{-1}$.

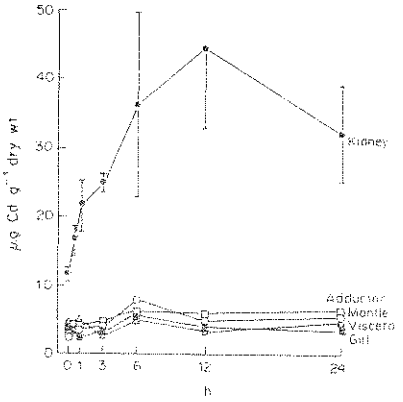

Fig. 2. Change of Cd concentration in various organs of *Anodonta cygnea* L. during 24 h exposure to 16 $\mu g\ Cd^{2+}\ l^{-1}$.

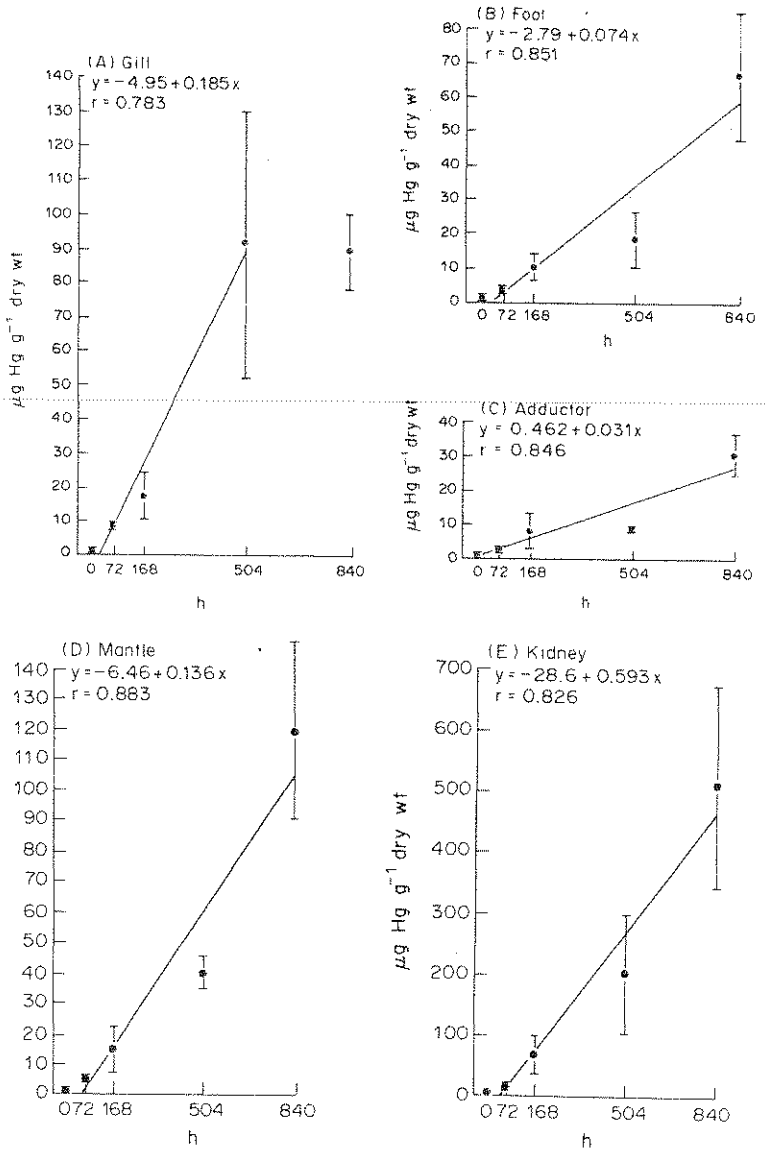

Fig. 3. Increase of Hg concentration in the gills (A), foot (B), adductor muscle (C), mantle (D) and kidney (E) of *Anodonta cygnea* L. during 72–840 h exposure to 10 μg Hg^{2+} l^{-1}.

1 h, and after 12 h exposure it was four times higher then in the control. This was followed by a moderate decrease up to 72 h.

Accumulation of Hg and Cd during long term exposure

The concentration of Hg increased linearly up to the end of the experiments (840 h) in nearly all organs. The highest Hg concentration was measured in the kidney, 512 ± 162 $\mu g\,g^{-1}$ (Fig. 3E). In the mantle, foot and adductor muscles there was also a linear uptake of Hg (Fig. 3D, B and C), however, after 504 h no further increase of Hg concentration was found in the gills (Fig. 3A).

The highest Cd concentration, 82.1 ± 24.7 $\mu g\,g^{-1}$ was measured in the kidney after 672 h (Fig. 4E), which decreased afterwards. Similarly, Cd concentration increased linearly up to 672 h in the viscera then dropped (Fig. 4F). In the mantle, gills and adductors the Cd uptake was linear by the end of the experiment (Fig. 4D, A and C).

The significance for the linearity shown in Figs 3 and 4 varies between $P < 0.001$ and $P < 0.01$.

Rate of bioconcentration

In the adductor muscles the factor of bioconcentration was close to 1000 for both metals after 336 h exposure and this value became higher for Hg by the end of the experiment. The mantle concentrated both metals with a factor of 1000 during the first 168 h. This value did not increase further for Cd however for Hg the factor became 10,000 during 840 h. In the gills the concentration factor for Cd reached 1000 by the end of the experiments, but for

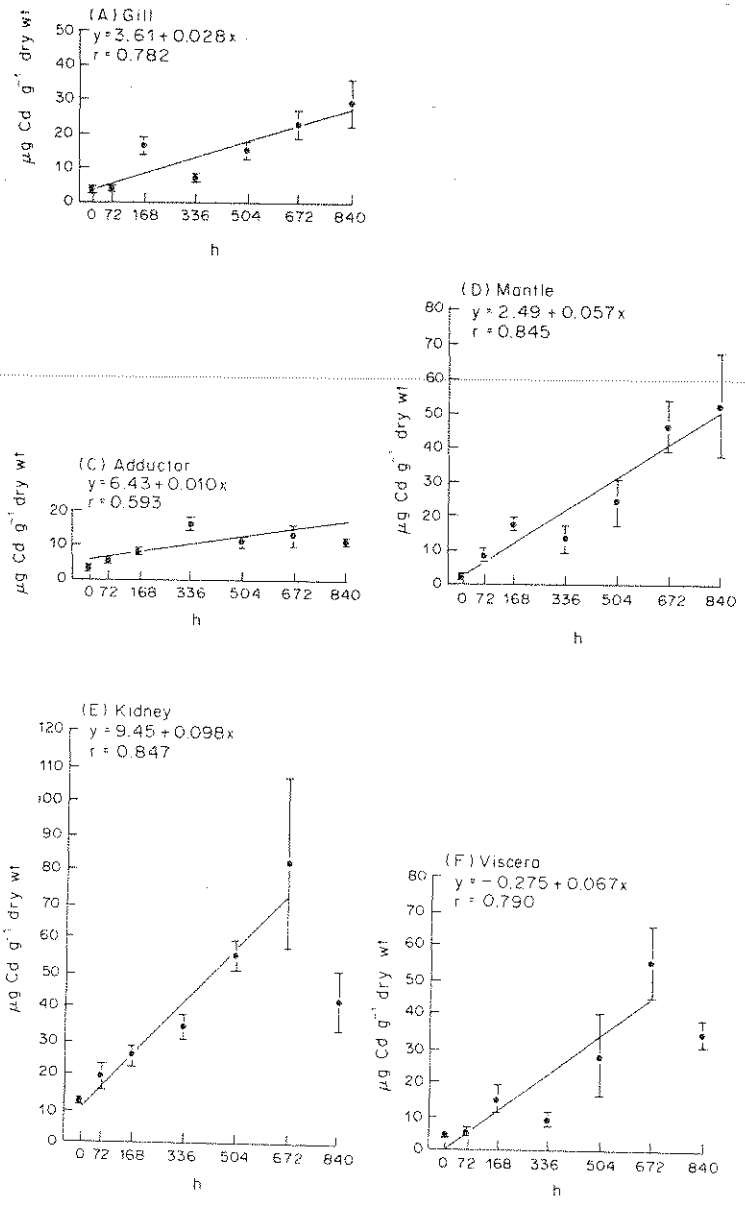

Fig. 4. Increase of Cd concentration in the gills (A), adductor muscle (C), mantle (D), kidney (E) and viscera (F) of *Anodonta cygnea* L. during 72–840 h exposure to 16 μg Cd^{2+} l^{-1}.

Hg it was close to 10,000 during 504 h. Kidneys showed a 1000 times concentration for Hg and Cd after 72 and 168 h respectively. By the end of the experiment the concentration factor for Cd did not reach 10,000, while for Hg it was found to be 10,000 at 504 h and nearly 100,000 at 840 h.

Change of the filtering activity

Under the influence of $HgCl_2$ and $CdSO_4$ the activity of the mussels changed remarkably. Before the application of the metals the length of the active periods varied between 10–25 h, but occasionally there were 48 h-long active periods. After exposure of the animals to heavy metals, the average duration of the active periods decreased to 5–10 h for several days, than gradually increased again and within 336 h it returned to approximately the control value (Fig. 5). At the same time the duration of the rest periods, which was 3–7 h in the control period, started to increase after adding $HgCl_2$ or $CdSO_4$ solution. The elongation of the rest periods resulted in a 3–7-fold increase by the end of the experimental period, and as a result the animals were in a closed position for about the same portion of the time as in activity (Fig. 6). Figures 5 and 6 show the duration of the subsequent active and rest periods respectively. Thirty consecutive periods are plotted for both periods, ten

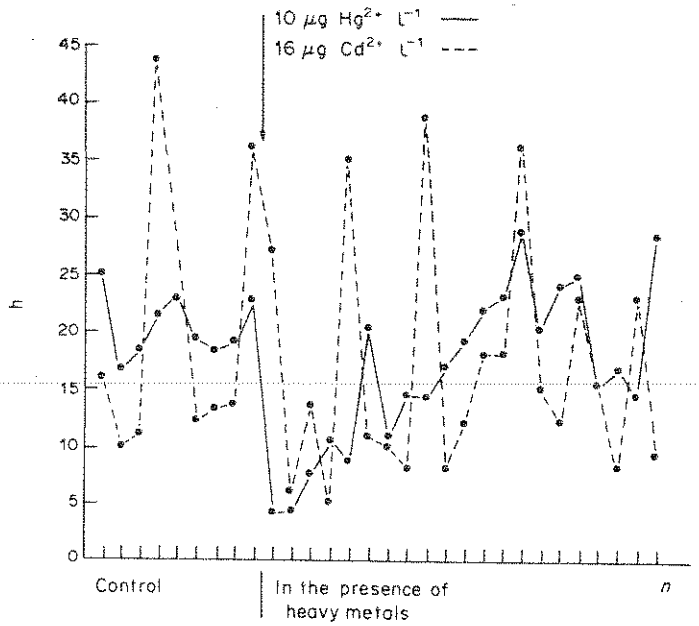

Fig. 5. Change of the duration (h) of consecutive active periods (n) as a result of exposure (arrow) to 10 μg Hg^{2+} l^{-1} and 16 μg Cd^{2+} l^{-1}.

before adding the metals and twenty afterwards. The change of the active periods is very similar for the Hg and Cd, but there is a difference in the rest periods for the two metals. Under the effect of Hg a gradual increase exists, however with Cd there is a periodical shortening and lengthening of the rest periods.

Change in weight of the tissues

We could follow directly the change of dry weight only in the case of Cd determination, because the method of Hg measurement requirement wet samples and in this case only indirect calculation was possible.

We found that under the influence of 16 μg l^{-1} concentration of Cd^{2+} there was a reduction in the weight of the tissues. Starting from 72 h of the experimental period when linear uptake of the metal begins, the dry weight of the gill, kidney, viscera, adductor muscles and mantle was reduced by 61, 50, 49, 43 and 21% respectively to the end of the exposure time (840 h). Under the influence of 10 μg l^{-1} Hg^{2+} a 19, 22 and 34% reduction of the wet weight was observed for the kidney, foot and adductor muscles

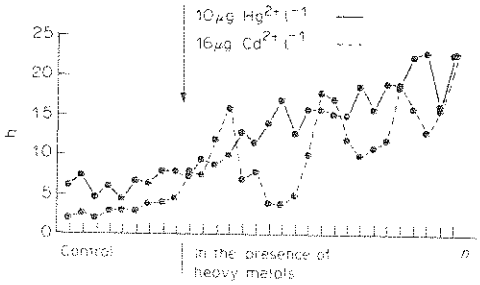

Fig. 6. Change of the duration (h) of consecutive rest periods (n) as a result of exposure (arrow) to 10 μg Hg^{2+} l^{-1} and 16 μg Cd^{2+} l^{-1}.

only. The dry weight of the gill, kidney, viscera, foot, adductor muscles and mantle was 0.16, 0.12, 0.12, 0.14, 0.13, 0.11 g g^{-1} wet wt, respectively.

DISCUSSION

Our results show that there is a difference between the Hg and Cd uptake by the fresh water mussel in the first day of exposure. The concentration of Hg increased in nearly all organs within 1 h, then with the exception of the kidney, it dropped below the control and reached the original level only after 24 h. However the concentration of Cd showed an increase in the first 24 h only in the kidney.

Investigating the Hg uptake in total wet tissue of *Crassostrea virginica*, Ruzic (1972) and Mason *et al.* (1976) described two independent phases of accumulation. According to them the first phase is a logarithmic, reversible, while the second is a linear, irreversible process. The transition from the first to the second phase occurs about 30–35 h for a 40 μg l^{-1} Hg concentration.

In our experiments we also found a non-linear and a linear phase of the uptake for Hg in all organs, however, for Cd uptake the two phase could only be distinguished in the kidney. The duration of the exposure during which the second phase prevails was between 24 and 72 h.

On the basis of these results it is suggested that there are differences in the mechanisms for the uptake and release of these two metals in various organs of *Anodonta* during the early period of exposure.

In accordance with the model of Ulfvarson (1962) when Hg release becomes much less then the uptake, the process of concentration can be characterized by

linear regression up to the saturation. In our experiments after 72 h both Hg and Cd uptake could be approximated with a simple linear regression (Figs 3 and 4) and most of the investigated tissues were not saturated up to the end of the experiment. However, the uptake for Hg was linear only up to 504 h in the gills and for Cd up to 672 h in the kidney and viscera. The metal concentration did not increase further with time in these organs at the given experimental conditions. These findings are in accordance with the data of Smith *et al.* (1975) who found that in *Anodonta grandis* the uptake of organic and inorganic Hg compounds was linear in a 4 day long experiment by the gills, foot and liver. Looking for Cd uptake in the total body of *C. virginica*, Zaroogian (1980) described a linear uptake in a 40 week long experiment when using 5, 10 and 15 $\mu g\ l^{-1}$ Cd concentration. In general our data are not in contradiction with these results, nevertheless, they show that in various organs the processes of uptake and storage can be different which is masked if measurements are carried out with total animals.

The speed of concentration of Hg and Cd is characterized during the linear period by the regression equations. As it is seen (Figs 3 and 4) accumulation of Hg was 6 times faster in the kidney and 4 times faster in the gills than accumulation of Cd. It is interesting to note that the uptake of Hg and Cd was 20 and 10 times faster into the kidney than in the adductor muscles.

During the experiments the highest concentration was found in the kidney after 672 h exposure. However, because of a decrease of Cd concentration in the kidney at the end of the experiment the order of organs was for Cd: mantle > kidney > viscera > gills > adductor muscles. For Hg it was the kidney which contained the highest concentration at each measurement. At the end of the experiment the order of organs was for Hg concentration: kidney > mantle > gills > foot > adductor muscles. These results show that in the bioconcentration process of heavy metals kidney plays an important role. This had been emphasized by Bryan (1976) and Carmichael *et al.* (1980). However, due to the low weight of this organ as compared to the total body weight, the kidney cannot be significant in the storage of Hg and Cd.

Capability for bioconcentration is considered as the main criterion for using an organism as a bioindicator (Van Esch, 1978). Our earlier investigations showed that the factor of bioconcentration of *Anodonta cygnea* is very high for Hg and Cd in natural environment. Its value was 1000 in the gills while in the foot, mantle and adductor muscles it varied between 100–1000 as compared to the water of the Lake Balaton (Salánki *et al.*, 1982). Recent determination (Table 1) showed that this value is even higher in the kidney where it surpasses 1000. According to the present experiments at higher metal concentrations the factor of bioconcentration was 10 times greater in the gills for both Hg and Cd, while in the mantle for Hg it was 100 times higher than in natural conditions. Since saturation was not reached in these organs one can suppose that the factor of concentration may increase over 10,000 during a long exposure or at higher metal concentrations.

In various tissues the Hg and Cd content changed. Taking into consideration the total mass of the investigated organs by the end of the experiments, the highest amount of Hg was present in the gills (181 μg), followed by the mantle (105 μg), foot (72.9 μg), kidney (37.3 μg) and adductor muscles (6.7 μg). The order of organs according to the Cd content was: mantle (42.3 μg), gills (39.8 μg), viscera (12.6 μg), kidney (4.63 μg), adductor muscles (2.52 μg). These data show that in *Anodonta cygnea* the gills and mantle can be used as best indicator organ for Hg and Cd pollution.

From factors influencing bioconcentration of water organisms the temperature (Pringle *et al.*, 1968) and the reproduction cycle are considered to be important (Cunningham and Tripp, 1973). Since Smith *et al.* (1975) did not find a significant difference in the accumulation of Hg between 10–20°C in *Anodonta grandis*, we did not take into consideration the change of t^0 in our experiments carried out between 7–15°C.

In the reproduction cycle there was no remarkable change during the experimental period. In winter there were, in some cases, growing veliger larvae in the gills. This could influence the increase of heavy metals in this organ, which could be monitored by special experiments. We did not follow the metal accumulation of the gills during spring, when glochidia are released to the environment.

In the evaluation of increased metal concentrations one should take into consideration the fact that during the experimental period the total weight of tissues was reduced by 30–50%. Supposing that the loss of weight is limited to the organic part of the body, it would itself be a factor increasing the metal concentration. However, on the one hand, the reduction of the body mass would increase the measured concentrations by 20–50% at the most, on the other, it is probable that together, with the loss of organic elements a part of the heavy metal is extruded from the animal. These considerations are supported by the results of Frazier (1975) who showed that the increase of Cd concentration in *C. virginica* cannot be explained by the reduction of body weight observed during Cd accumulation.

As a result of exposure to Hg and Cd the filtering activity of the mussels was reduced. Measuring the length of activity and rest it was clear that the proportion of the activity became less as compared to the control. The elongation of the rest periods when shells are tightly closed causes the decrease in feeding time, which can be one of the factors causing a weight loss, however this can also reduce uptake of metals. At the same time the transport and redistribution as

well as binding of the metals already inside the animal can take place in closed shells as well. The duration of consecutive active and rest periods varies differently during Hg and Cd uptake (Figs 5 and 6) referring again to the fact that there is a difference not only between the uptake mechanism but also between the physiological effect of these two heavy metals.

At higher Hg and Cd concentrations as well as other heavy metals, the filtering activity of bivalve molluscs became strongly reduced (Salánki, 1966; Salánki and Varanka, 1976). This can have a marked effect on the bioaccumulation. The mechanisms which heavy metals influence, is still unknown. As in the neural regulation of the activity and rest of mussels, serotonin and catecholamines play a key function (Salánki *et al.*, 1974; Hiripi, 1977) one can suppose that the effect of heavy metals may be realized through acting on the metabolism and release of these neurotransmitters in the brain or at the periphery.

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