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CADMIUM INDUCED CHANGES OF THE METABOLIC ACTIVITY OF THE FRESHWATER SNAIL *Planorbis corneus* ESTIMATED BY DIRECT CALORIMETRY

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By use of flow-microcalorimetric studies the effect of cadmium in the sublethal concentration range (0.5-h LC_{50} is 1.69 mg/l) on the metabolic activity of the freshwater snail *Planorbis corneus* was revealed. The high toxicity was confirmed by the change in the heat production rate (mean value is 453 μW/g fresh weigth) in a cadmium perfusate down to a concentration of 0.01 μg/l. The decrease in heat output depended on the cadmium concentration and amounted from 19% at 0.01 μg/l to 88% at 1000 μg/l. The microcalorimetric method proved to be a useful continuous-flow technique to assess qualitatively and quantitatively the toxic effects of cadmium on an aquatic organism already 4 hours after exposure.

Introduction

Mankind has become the most important element in the global biogeochemical cycling of the trace metals [1]. There is a significant contamination of freshwater resources and since heavy metals are not degradable an accelerating accumulation in the human food chain takes place.

Among the heavy metals cadmium (Cd-compounds and dust) seems to be toxic to all life forms [2, 3] because of its highly oxidative and denaturating effect on protein. Cadmium has no known physiological role and is generally accumulated by organisms

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[4], in vertebrates as metallothionein [5, 6]. In 1983 the anthropogenic input of cadmium into water amounted to 17000 kg [1] from which the average concentration in lakes and rivers would be increased by about 0.18 µg/l. The background concentration in unpolluted lakes and rivers is below 1 µg/l [3,7]. For several organisms the lethal threshold concentration is 1 µg/l in freshwater and 100 µg/l in seawater [8]. For aquatic and marine organisms LC_{50}-values between 10 and 100 µg/l (depending on exposure time, temperature and salinity) are reported [9 - 17].

Among the bioassay methods for the examination of water and and sewage sludge the most frequently used techniques are mortality studies {lethal-dosis (LD) or lethal-concentration (LC) tests} over a given period of time. Sublethal effects are often more difficult to assess and much more protracted [18] than immediate lethality, but more sensitive to potential hazards of low levels of toxic material. Sublethal pollution can modify physiological or behavioural responses of organisms [19]. Therefore the energy metabolism as the whole of all biochemical processes within a living system should reflect the impact of sublethal environmental stresses.

The metabolic rate (energy demand) can be estimated indirectly from the rate of oxygen consumption or directly from the rate of heat production. Calorimetry can thus be used as an analytical method to follow the course of a reaction, with the rate of heat production being proportional to the intensity of the reaction, and the amount of exchanged heat being proportional to the extend of the process. Direct calorimetry operates integrally since at any time all heat effects occuring in the sample, including side effects and unexpected effects, give one summarized thermic signal [20].

In the following paper microcalorimetry is presented as a sensitive biotic monitoring method by which very small effects of cadmium on a limnic organism are described. The freshwater snail <u>Planorbis</u> <u>corneus</u> was chosen as test organism because it is widespread over Europe in stagnant waters and because of its ecologic importance as an invertebrate in the beginning of the food chain [21].

Materials and methods

Calorimeter

The heat production of the snails was determined by a sorption microcalorimeter (LKB, Sweden, type 2107-122) at 21°C. The size of the sorption vessel was enlarged to a height of 26 mm and a diameter of 12 mm, so that animals up to 600 mg fresh weight could be examined. The sensitivity of the calorimeter was 38 mV/W . For a calorimetric run one snail was placed in the sorption vessel, which was filled with water, inserted into the calorimeter and connected to the flow system (Fig. 1). The pump velocity through the calorimetric chamber was 9.5 ml/h, a flow which produced a thermal noise of 90 µW and which did not cause the animals to draw back in their shells. When the water was changed to the desired Cd-concentration a velocity of 60 ml/h was run for 10 minutes to accelerate mixing. The enhanced flow rate led to a typical series of successive endothermic and exothermic peaks in the heat output, each of a 100 µW displacement.

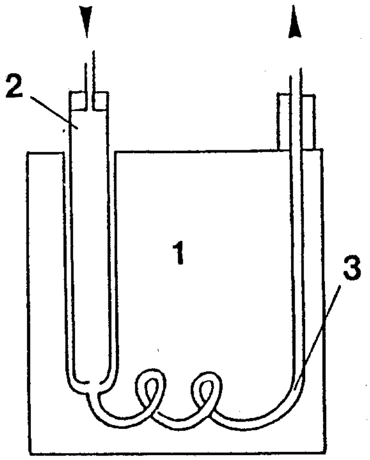

Fig.1: Schematic drawing of the calorimetric unit: 1. Heat sink and measuring device, 2. calorimetric chamber, 3. heat exchanger. The arrows indicate the flow direction.

After 30 minutes of thermal equilibration the evolved thermovoltage was amplified and recorded (Linseis L 1040, Germany) as a power-time-curve $dQ(t)/dt$ over 4 to 5 hours until the heat signal was sufficiently stable. Then the perfusion water was changed to the desired cadmium concentration. The heat production of the snail under exposure of Cd-solution was recorded over another 4 h-period. After each experiment the snail was removed for weighing and the calorimetric reference line was determined by pumping the same Cd-solution through the flow system as under the experiment.

Organism

The freshwater snails <u>Planorbis corneus</u> LINNE 1758 were obtained from an aquarist shop and kept in an aerated aquarium at 21 °C using a 12 h-photoperiod. The water used during acclimatization and during the experiments was normal tap water, which was allowed to settle for 3 days (pH 7.4 to 7.7). Ten days before a calorimetric experiment the water was renewed and feeding was stopped. After 5 to 6 days defecation was completed. The animals were selected to be within a narrow size range.

Toxicity test

Cadmium stock solution was prepared by dissolving analytical grade $CdCl_2 \cdot 2\frac{1}{2}H_2O$ in deionized water at 10 mg/l. The stock solution was added to the water to achieve the desired concentration. All results refer to standard deviations of at least 5 individuals analysed separately.

Results

Data on the toxicity of cadmium on <u>Planorbis corneus</u> are given in [22] . From these data an acute 0.5-h LC_{50}-value (median lethal value of initial concentration of test solution) of 1.69 mg/l with a 95 % confidence limit between 0.69 and 2.69 mg/l is computed. After exposure intervals up to 32 h about

90 % of the incorporated Cd was found in the back part of the body, about 6 % in the forebody and about 4 % in the shell. For the calorimetric experiments Cd-concentrations below this value were chosen. Fig.2 shows the course of the heat production (power-time-curve) of five different snails kept in normal non-contaminated water. During the first four hours there are short-term fluctuations of heat dissipation but then the heat signal becomes more stable and amounts to (453 ± 80) µW/g of fresh weight (n = 22). These values were corrected by the background level of 90 µW produced by the heat due to the friction of the flow.

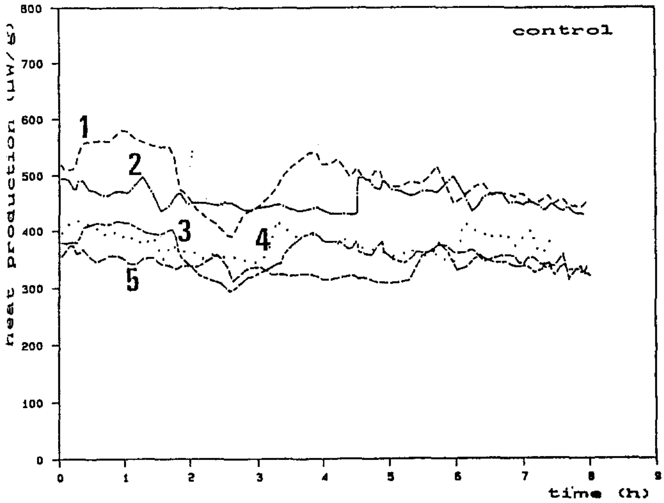

Fig.2: Time course of the heat production rate of five individual snails in normal water.

The time for changing the flow from water to the desired cadmium solution was chosen at 4.5 hours after start of the experiment.This time is indicated by an arrow in Fig. 3 which shows the power-time-curves of cadmium-exposed animals. A more disturbed pattern of heat production appears upon exposure. In all cases a distinct fluctuation over about 30 minutes occured followed by a plateau of different length and a subsequent decrease in the heat dissipation to another final plateau. Generally after 8 hours the experiment was interrupted and the animals were examined for locomotor activity. After an exposure time of 3.5 hours with the highest concentration (1 mg/l) 75 % of the animals failed any locomotory response upon mechanical stimuli. In all other cases and concentrations responses were ascertained.

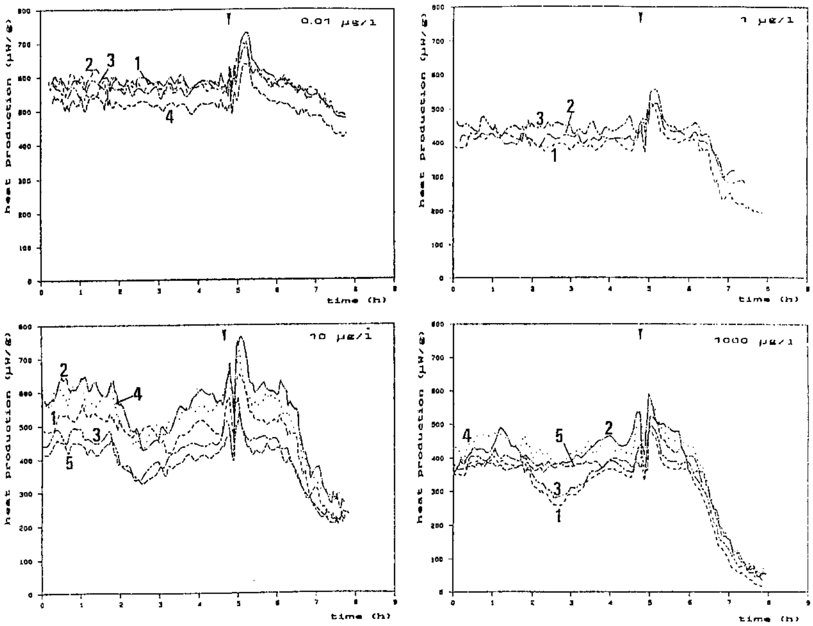

Fig.3 : Time course of the heat production rate of cadmium exposed snails. The arrows indicate the start of exposure.

Discussion

Unexposed animals

From Fig. 2 it is obvious that the power-time-curves differ in their absolute value as well as in their profile. The short-time fluctuations are presumably due to stress reactions and subsequent locomotor activities of the animals when being put into the calorimetric chamber [23, 24]. After an adaptation period of 4 hours the heat signal stabilizes and continues with a slight decrease over more than one day. The average value of about 450 µW/g fresh weight at 21°C representing the basal metabolism of a resting animal is higher than those of 110 µW/g at 28°C and 220 µW/g at 18°C for the same organism determined in a batch calorimeter [25] and lower than the values of 450 to 1650 µW/g at 21.8°C for the snail Littorina littorea [26, 27] and 890 to 1020 µW/g at 27°C for the snail snail Biomphalaria glabrata [28, 29]. The relative broad standard deviation of ±80 µW/(g fresh weight) may be partially attributed to the difference between the total fresh weight and the weight of the soft tissue (total weight minus shell weight) of an animal which comes up to about 55 % . The thermodynamic values given here refer to the total fresh weight, whereas heat is evolved mainly by the metabolic active tissue, the weight of which is correlated to the total weight by a coefficient (Pearsons correlation factor) of only 0.9056 [22].

Cd-exposed animals

Upon Cd-exposure the power-time-curves of the snails exhibit in all individual cases and at all concentrations a similar behaviour which is characterized by an immediate short fluctuation, a more or less constant shoulder and an decrease to a stable final value. Whereas the first phase is due to thermal disturbances and mixing effects the following shoulder and decline represent the interference of cadmium with the organism. Table 1 shows the length of the shoulder plateau, the decline of the heat production given as relaxation time (time interval during which the heat production drops to 1/e ≈ 37% of its initial value) and the percent decrease three hours after exposure. All these parameters depend on cadmium concentration.

Since the mode of cadmium action is certainly a complex one and cannot be described by simple chemical reaction kinetics, the parameters of Table 1 represent only unspecific phenomenological responses and can not be attributed to single chemical

Table 1: Mean values of three parameters of the decreasing part of the power-time-curve after exposure to cadmium.

cadmium concentration (µg/l)	length of plateau (h)	relaxation time of decline (h)	percentage of initial value after 8 hours (%)
0	∞	9.8	90
0.01	2.5	2.3	81
1	2.0	1.3	54
10	1.8	0.7	47
1000	1.5	0.4	12

or metabolic events. But nevertheless they present themselves as very sensitive indicators which are perceptible even at low concentrations (0.01 µg/l) and are at hand already 8 hours after starting the experiment or 4 hours after exposure. Just from this last example and from the 1 µg/l-experiments it can be concluded, that the lethality threshold of 1 µg/l for cadmium reported by Merian [8] is too high, though the restrictive conditions within a calorimetric chamber seem to play a role.

In spite of clear differences in the heat production curve of the individuals a parallelism in the reaction of these animals after exposure is generally noticed. The effect of cadmium is evidently so intense and characteristic that no "freak values" occur. In comparison to the normally used lethality tests the calorimetric method needs a smaller sampling size.

Further investigations are necessary to explain the effects to which the final heat output following the decrease of heat production after exposure can be attributed to. This end-value is generally reached 3.5 to 4 hours after exposure but on different levels depending on the cadmium concentration. Except with the highest concentration of 1000 µg/l the animals were not dead at this time.

References

1 J.O. Nriagu and J.M. Pacyna, Nature 333 (1988) 134.

2 M. Cooke, A. Jackson, G. Nickless and D.J. Roberts, Bull. Environm. Contam. Toxicol. 23 (1979) 445.

3 L. Friberg, G.F. Nordberg and V.B. Vouk (eds.); Handbook on the Toxicology of Metals, Elsevier/North-Holland Biomedical Press, Amsterdam (1979).

4 R.J. Mirenda, Arch. Environ. Contam. Toxicol. 15 (1986) 401.

5 J.H.R. Kägi and M. Nordberg (eds.), Metallothionein, Experientia Suppl. 34, Birkhäuser Verlag, Basel (1979).

6 M. Karin, A. Haslinger, H. Holtgreve, R.I. Richards, P. Krauter, H.M. Westphal and M. Beato, Nature 308 (1984) 513.

7 J.M. Martin, G.A. Knauer and A.R. Flegel, in: Cadmium in the Environment (ed. J. O. Nriagu), John Wiley, New York (1980), part I, pp. 141.

8 E. Merian, Metalle in der Umwelt, Verlag Chemie, Weinheim (1984).

9 V. Axiak and J.L. Schembri, Mar. Pollut. Bull. 13 (1982) 383.

10 K.E. Biesinger and G.M. Christensen, J. Fish. Res.Board Can. 29 (1972) 169.

11 R. Eisler and R.J. Henneky, Arch. Environm.Toxicol. 6 (1977) 315.

12 W.S. Hall, R.L. Paulson, L.W. Hall and D.T. Burton, Bull. Environm. Contam. Toxicol. 37 (1986) 308.

13 M.W. Johnson and J.H. Gentile, Bull. Environm. Contam. Toxicol. 22 (1979) 258.

14 J.S. Marshall, J. Fish. Res. Board Can. 35 (1978) 461.

15 M. Moraitou-Apostolopoulou, G. Verriopoulos and P. Lentzou, Bull. Environm. Contam. Toxicol. 23 (1979) 642.

16 R.W. Winner, Aquat. Tocicol. 5 (1984) 267.

17 D.A. Wrigth and J.E. Frain, Environm. Res. 24 (1981) 338.

18 W.B. Vernberg, P.J. DeCoursey, M. Kelly and D.M. Johns, Bull. Environm. Pollut. Toxicol. 17 (1977) 16.

19 P.J. DeCoursey and W.B. Vernberg, Oikos 23 (1972) 241.

20 C. Spink and I. Wadsö, Calorimetry as an Analytical Tool in Biochemistry and Biology, in: Methods in Biochemical Analysis (ed. D. Glick), vol. 23, Wiley Interscience, New York (1976) pp. 1-159.

21 H.D. Maciorowski and R. Mc V. Clarke, Advantages and Disadvantages of Using Invertebrates in Toxicity Testing in: Aquatic Invertebrate Bioassays, ASTM STP 715 , American Society for Testing and Materials (1980) 36-47.

22 G.B. Joachimsohn, Mikrokalorimetrische Untersuchungen der akuten Wirkung von Cadmiumchlorid auf den Süßwasserpulmonaten Planorbis corneus LINNE 1758, Doctoral Thesis (1987) Freie Universität Berlin.

23 G.B. Joachimsohn, I. Lamprecht and K. Graszynski, Thermochim. Acta 94 (1985) 151.

24 I. Lamprecht and W. Becker, Thermochim. Acta 130 (1988) 87.

25 H. Kinne, Manometrische und Mikrokalorimetrische Untersuchungen zur Temperatur- und Größenabhängigkeit des Stoffwechsels des Süßwasserpulmonaten Planorbis corneus L., Diplomarbeit (1986), Fachbereich Biologie, Freie Universität Berlin.

26 C.S. Hammen, Comp. Biochem. Physiol. 62A (1979) 955.

27 C.S. Hammen, Comp. Biochem. Physiol. 67A (1980) 617.

28 W. Becker and I. Lamprecht, Z. Parasitenk. 53 (1977) 297.

29 W. Becker, J. Comp. Physiol. 135 (1980) 101.

Zusammenfassung

Mit Hilfe der Flow-Mikrokalorimetrie wurde die Wirkung von Cadmiumchlorid im subletalen Konzentrationsbereich auf die Stoffwechselaktivität der Süßwasserschnecke Planorbis corneus untersucht. Die Wärmeproduktion der Tiere sank im Mittel von 435 µW/g Frischgewicht bei einer Cadmiumkonzentration von 0.01 µg/l auf 81 % und bei 1000 µg/l auf 19 %. Die Kalorimetrie erwies sich als eine nützliche Flow-Technik, mit der die Toxizität von Cadmium qualitativ und quantitativ bereits 4 Stunden nach der Exposition erfaßt werden kann.

Резюме – С помощью микрохалориметра с тепловым потоком оценено влияние кадмия в сублетальной концентрации /LC_{50} = 1,69 мг/л/ на метаболическую активность пресноводной улитки Planorbis corneus . Высокая токсичность кадмия была подтверж-

дена путем измерений изменения скорости выделения тепла / среднее значение составляло 453 μВт на грамм живого веса / при опрыскивании раствором кадмия до концентрации 0,01 μг/л. Уменьшение выделения тепла зависит от концентрации кадмия и количественно изменяется от 19% при концентрации кадмия 0,01 μг/л до 88% при концентрации 1000 μг/л. Показано, что микрокалориметрия непрерывного потока является приемлемой для количественного и качественного определения токсичного действия кадмия на водообитающие организмы уже после четырехчасового воздействия.