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Effect of Selenite on the Uptake of Methylmercury in Cod (*Gadus morhua*)

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A protecting role of selenite against the toxic effects of different mercury compounds is well documented. The organ distribution and retention of inorganic mercury as well as of methylmercury seem to be influenced by a high selenium status. Several reports have confirmed these effects for a wide variety of vertebrates such as mammals (Burk et al. 1974; Mengel and Karlog 1980), birds (Ganther et al. 1972) and fishes (Kim et al. 1977; Heisinger et al. 1979).

Mercury is characteristically present in the aquatic environment and follows the food chain. An examination of the species at the top of this chain is of interest as it is within these groups of animals that the highest concentrations of the elements have been reported (Koeman et al. 1975; Mackay et al. 1975). Several authors have shown a correlation between high concentrations of mercury and selenium in aquatic organisms (Schultz and Ito 1979; Luten et al. 1980). Virtually nothing is known about the interaction of methylmercury and selenium in feeding experiments on fish. Therefore the present study was designed to determined the possible interaction of methylmercury and selenium in different tissues of cod (*Gadus morhua*), and important foodfish, feeding diets supplemented with nontoxic levels of the elements.

MATERIALS AND METHODS

Cod (*Gadus morhua*) weighing between 100 and 400 g were obtained from the Masfjord in Western Norway. They were acclimatized in a sheltered tank supplied with running sea water at a temperature of 8°C and a salinity of 35 o/oo. After an adaptation period of one month fish were randomly divided into four groups in aquaria of 125 l. The photoperiod was automatically regulated to 12 h light and 12 h dark, and the other parameters were the same as during acclimatization.

Wet-feed based on capelin (*Mallotus villosus*) was prepared every second day. The feeds were ground, 2% of a vitamin mixture added, and finally they were sprayed with solutions of the dissolved supplements of methylmercury and selenium.

The methylmercury was added as a solution of CH_3HgI (1.0 g/l) dissolved in acetone. Selenite was added as a solution of SeO_2 in water, corresponding to 1.0 g Se^{4+}/l. The feed was formed as small pellets and the fish fed *ad libitum* twice daily.

At the beginning of the experiment ten fish were randomly selected from the acclimatized stock to establish baseline values for mercury and selenium. Four groups, each containing 10-15 fish randomly selected, formed the experimental groups. The total experiment period lasted for 32 days with sampling after 8, 16 and 32 days. At the end of the trial five fish were sampled from the remaining stock which had been fed a diet without supplementations, these were used as controls. The experimental plan is given in Table 1.

Table 1. Experimental plan for mercury and selenium supplementation.

Group	Supplementation ($mgkg^{-1}$ wet feed)	Analysis ($mgkg^{-1}$ feed)	
		Mercury	Selenium
CONTROL		<0.02	0.12
1	CH_3Hg (2.5)	2.06	0.12
2	CH_3Hg (2.5) + Se(IV) (2.5)	2.17	2.59
3	CH_3Hg (2.5) + Se(IV) (5.0)	2.20	5.16
4	Se(IV) (5.0)	<0.02	5.03

Each sampled fish was weighed and dissected to give samples of the dorsal muscle, liver and brain. The samples of muscle and liver tissue were prepared from each fish, whereas the brain tissues from each group were pooled. The muscle and brain tissues were freeze-dried to a constant weight, homogenized and stored in tightly closed bottles. The liver samples were homogenized and stored in closed bottles at -20°C.

Table 2. Concentration of mercury and selenium ($mgkg^{-1}$ wet weight) in muscle tissue of cod, *Gadus morhua*, exposed to methylmercury and selenium in the diet, either separately or simultaneously, Means ± standard deviation (Number of analysis in parenthesis).

Group	Supplementation ($mgkg^{-1}$ wet feed)	Day 0	Day 8	Day 16	Day 32
Total mercury concentration					
	CONTROLS	0.03±0.01(10)	n.d.[b]	n.d.[b]	0.03±0.01(5)
1	CH_3Hg (2.5)	0.03±0.01(10)	0.15±0.02(5)	0.37±0.08(5)	0.79±0.08(4)
2	CH_3Hg (2.5) + Se(IV) (2.5)	0.03±0.01(10	0.10±0.02(3)	0.25±0.02(3)	0.88±0.06(3)
3	CH_3Hg (2.5) + Se(IV) (5.0)	0.03±0.01(10)	0.16±0,06(3)	0.50±0.16(4)	0.89±0.21(4)
Total selenium concentration					
	CONTROLS	0.14±0.02(10)	n.d.[b]	n.d.[b]	0.15±0.02(5)
3	CH_3Hg (2.5) + Se(IV) (5.0)	0.14±0.02(10)	0.26±0.01(3)[a]	0.25±0.04(4)	0.29±0.02(4)[a]
4	Se(IV) (5.0)	0.14±0.02(10)	0.21±0.01(3)[a]	0.23±0.02(3)	0.34±0.03(4)[a]

[a] Groups significantly different at $P < 0.05$ - Fischer "F" test.
[b] Not determined.

The digestion procedure was as described by Julshamn et al. (1982b). Samples of 0.1-0.2 g of freeze-dried material or of the homogenized liver tissue were weighed into 20 ml Sovirel bottles. 1.8 ml conc. nitric acid and 0.2 ml conc. perchloric acid were added to the samples which were preashed over night. The bottles were sealed and heated in a commercial household pressure boiler at 106°C (0.5 atm. above atmospheric pressure) for 2 hours. The solutions were then diluted to 10 ml with distilled deionized water. Both elements were analysed in the same digest.

Mercury was analyzed with a Perkin-Elmer Model 370 Atomic absorption spectrophotometer (AAS) equipped with a Perkin-Elmer Mercury Analysis System and an EDL as light source. The measurement was carried out at 253.7 nm with a slit width of 0.2 nm. The procedure used is described by Egaas and Julshamn (1978). Standard addition was used as an intermediate control of possible chemical interferences in the analysis (Julshamn and Braekkan, 1975).

The selenium analysis was carried out as described by Julshamn et al. (1982b). A Perkin-Elmer Model 5000 AAS equipped with a HGA-76 Graphite Furnace was used. A selenium EDL-source with a 196.0 nm resonance line, a 2.0 nm slit and a Deuterium Background Corrector were used in all determinations. Nickel nitrate was used as a heat stabilizing reagent and the temperature program was as follows: Drying 120°C (30 sec.), Ramp Charring 120°C-600°C (45 sec.), Charring 600°C (30 sec.) and Atomization 2650°C (3 sec.). Standard addition was used in all determinations (Julshamn and Braekkan, 1975).

RESULTS AND DISCUSSION

The mercury and selenium concentrations in the investigated organs of the control fish did not change during the experiment (Table 2, 3 and 4).

There were no significant differences ($P < 0.05$) in the mercury uptake in the muscle between the groups with methylmercury exposure (group 1, 2 and 3; Table 2). The mercury concentration in muscle increased steadily in all three groups during the whole experimental period. The selenium supplement therefore did not seem to influence the mercury uptake, quantitatively or with regard to the uptake rate in the muscle. The selenium uptake in muscle was significantly higher in group 3 at day 8 and lower at day 32 compared with group 4, probably a result of a different uptake rate in group 3, which shows a saturation at about 0.26 $mgkg^{-1}$ after 8 days in group 3. The group fed solely selenium (group 4) had a significant increase ($P < 0.05$) in

Table 3. Concentration of mercury and selenium ($mgkg^{-1}$ wet weight) in liver tissue of cod, *Gadus morhua*, exposed to methyl mercury and selenium in the diet, either separately or simultaneously, Mean ± standard deviation (Number of analysis in parenthesis).

Group	Supplementation ($mgkg^{-1}$ wet feed)	Day 0	Day 8	Day 16	Day 32
Total mercury concentration					
CONTROLS		0.04±0.01(9)	n.d.[b]	n.d.[b]	0.05±0.02(5)
1	CH_3Hg (2.5)	0.04±0.01(9)	0.62±0.13(5)	0.59±0.17(4)	0.65±0.08(4)
2	CH_3Hg (2.5) + Se(IV) (2.5)	0.04±0.01(9)	0.42±0.10(3)	0.70±0.03(3)	0.96±0.18(4)
3	CH_3Hg (2.5) + Se(IV) (5.0)	0.04±0.01(9)	0.38±0.10(3)	0.57±0.13(4)	0.84±0.15(4)
Total selenium concentration					
CONTROLS		0.18±0.06(9)	n.d.[b]	n.d.[b]	0.16±0.07(5)
3	CH_3Hg (2.5) + Se(IV) (5.0)	0.18±0.06(9)	0.46±0.03(3)	0.78±0.09(4)[a]	0.86±0.10(4)[a]
4	Se(IV) (5.0)	0.18±0.06(9)	0.57±0.06(3)	0.56±0.03(2)[a]	0.58±0.06(4)[a]

[a] Groups significantly different at $P < 0.05$ - Fischer "F" test.
[b] Not determined.

the selenium concentration from day 8 through day 16 to day 32.These differences in the selenium uptake are very small, and with no differences in the mercury concentration there seems to be no clear interaction of methylmercury and selenium in the muscle tissue of cod. These results are consistent with the findings of Luten et al. (1980), who found no relation between the mercury and selenium concentrations in the fillets of 53 cod samples caught in the sea. Cappon and Smith (1981), neither found any correlation between the muscle selenium and mercury concentrations in several species of fish, but there are other reports on such cirrekatuibs in fish muscle (Mackay et al. 1975; Shultz and Ito 1979).

Among the three tissues analysed, the muscle accumulated the highest concentrations of mercury during the 32 days of exposure without selenium supplementation (group 1; Table 2, 3 and 4). This effect is of interest because other heavy metals such as copper, zinc, cadmium and lead all have higher liver concentrations compared with the muscle tissue in cod (Julshamn and Braekkan 1975).

The mercury concentration in the liver in group 1 increased to approximately 0.62 $mgkg^{-1}$ after 8 days, and this level was constant through the rest of the experimental period (Table 3). Both group 2 and 3 given selenium supplementation increased significantly from day 8 to day 32 ($P < 0.05$). The selenium uptake was significantly higher after 16 and 32 days in group 3 compared with group 4. The group with selenium supplementation alone (group 4) reached a level of approximately 0.57 $mgkg^{-1}$ after 8 days. In group 3, with methylmercury supplementation, the selenium concentration increased throughout the whole experimental period. This effect was the same as observed for the mercury uptake in the liver. Although the feeding of methylmercury and selenium gave small quantitative differences between the groups, the interaction of the elements are shown in the different uptake pattern in the liver. Separate intake of the elements led to a saturation of the uptake after only 8 days. Simultaneous intakes increased the uptake for both elements over a longer range of time (at least 32 days). The increased liver uptake of mercury and selenium when fed simultaneously is well documented in mammals (Mengel and Karlog 1980; Komsta-Szumska 1983). This effect is also shown in the correlation of the elements in the liver of different fish species (Mackay et al. 1975; Shultz and Ito 1979; Luten et al. 1980). The liver may therefore be a site for the selenium detoxification of mercury in fish.

Table 4. Concentration of mercury and selenium ($mgkg^{-1}$ wet weight) in brain tissue of cod, *Gadus morhua*, exposed to methylmercury and selenium in the diet, either separately or simultaneously, values for pooled samples from (N) individuals are shown in parenthesis.

Group	Supplementation ($mgkg^{-1}$ wet feed)	Day 0	Day 8	Day 16	Day 32
Total mercury concentration					
CONTROLS		0.03(10)	n.d.[a]	n.d.[a]	0.03(5)
1	CH_3Hg (2.5)	0.03(10)	0.28(5)	0.53(5)	0.67(5)
2	CH_3Hg (2.5) + Se(IV) (2.5)	0.03(10)	0.50(3)	0.55(3)	1.43(3)
3	CH_3Hg (2.5) + Se(IV) (5.0)	0.03(10)	0.42(3)	0.79(4)	1.81(4)
Total selenium concentration					
CONTROLS		0.23(10)	n.d.[a]	n.d.[a]	0.24(5)
3	CH_3Hg (2.5) + Se(IV) (5.0)	0.23(10)	0.23(3)	0.39(4)	0.43(4)
4	Se(IV) (5.0)	0.23(10)	0.25(3)	0.25(3)	0.37(4)

[a] Not determined.

The total uptake of mercury in the brain increased with the selenium supplementation (Table 4). Already after 8 days this effect was clear, and after 32 days the uptake was between 2 and 3 times higher in group 2 and 3 compared with group 1. The selenium uptake seemed not to be influenced by the methylmercury supplementation in group 3. The use of pooled samples excluded statistical evaluation, but the increased mercury uptake with selenium supplementation was substantial. As a similar effect of methylmercury on the selenium uptake was not found, the interaction of methylmercury and selenium in the brain was different from that in the liver. There is little information available on this interactive effect in the brain tissue of fish, and a comparison must be made with mammals. An elevated level of mercury in the brain has been recorded in mice and rats when methylmercury and selenium were fed simultaneously (Iwata et al. 1973; Chen et al. 1975; Mengel and Karlog 1980). This increased methylmercury uptake to the nervous system seems contradictory to the protective action of selenium against methylmercury, since methylmercury itself is a strong neurotoxin. Magos and Webb (1980) have discussed this effect in a review, but their main conclusion was that the protective effect of selenite against methylmercury is not the result of a decrease in the methylmercury concentration in the nervous system, on the contrary an increase is often recorded.

Although our feeding experiments can vige no information on the mechanism of a methylmercury-selenium interaction in cod, the results indicate such an effect through the altered uptake of these elements in the liver and the brain. Other reports have shown an increased whole body retention of mercury in fish exposed simultaneously to inorganic mercury and selenium (Kim et al. 1977; Heisinger et al. 1979). Therefore, there seems to be an interaction of inorganic as well as organic mercury in fish, but inorganic mercury is poorly absorbed from the feed (Julshamn et al. 1982a) and may not be critical to the fish.

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