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STUDIES IN SEARCH OF MODIFIERS OF THE TOXICITY OF MERCURIALS AND SPECULATIONS ON ITS BIOCHEMICAL MECHANISM

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Abstract—Experiments were conducted to ascertain if exposure of goldfish (*Carassius auratus*) to L-cysteine, L-glutathione, calcium pantothenate, pantethine, or coenzyme A modifies the acute toxicity of mercuric and methylmercuric chlorides. It was found that exposure to L-cysteine, together with a mercurial, protected all the fish despite substantial accumulations of mercury by them. Coenzyme A was effective against methylmercury when exposure was simultaneous with the mercurial, while it was effective against mercuric chloride when exposure was 24 hr before exposure to the mercurial. These observations are consistent with our hypothesis that at least part of the toxic action of mercury is due to its combination with coenzyme A.

It is well established that the mercurials have an exceptionally high affinity for sulfhydryl groups [1, 2]. In biological systems, sulfhydryl groups are found in a few diffusible low molecular weight substances, such as cysteine, reduced glutathione, coenzyme A, lipoate and thioglycolate, but sulfhydryl groups are predominantly constituents of proteins [3]. It is argued that, if any of these sulfur compounds is vital to the organism, it would also modify toxic effects of mercury compounds. Therefore in the present study an attempt has been made to find out if these compounds reduce the toxicity of mercuric and methylmercuric chlorides, which were selected because they are present in the aquatic environment and food chains and are of toxicological interest.

MATERIALS AND METHODS

The details about the goldfish (*Carassius auratus*), aquariums, and radioactive mercury compounds have been described earlier [4]. The effects of L-cysteine (BDH), reduced L-glutathione (Sigma), and coenzyme A (CalBiochem) on the acute toxicity of both mercuric and methylmercuric chlorides were determined in a series of experiments with different application regimens. Calcium pantothenate (CalBiochem) and pantethine (CalBiochem) were also used in view of the significant protective effects observed with coenzyme A.

For each of the mercury compounds there was one control and eight experimental tanks of 10 litre capacity. Each tank had ten goldfish, with an average total length of 5.5 cm. In the control tank only the radioactive mercury compound was added in the concentration previously used [5]. In the experimental tank the same amount of labelled mercury compound was added, together with a suitable amount of modifier, and the uptake of both was allowed to take place simultaneously for 24 hr. In another application regimen, the fish were first immersed for 24 hr in a tank containing modifier and then exposed to the mercury compound for the next 24 hr in another tank.

At the end of 24 hr of mercury uptake the whole fish were counted for gamma activity due to ^{203}Hg in a Packard Auto-Gamma Scintillation Spectrometer model 5130 as done before [5]. The results were assessed both on the basis of acute toxicity experiments as determined by 24-hr survival rates and of the effect of treatment on the uptake of mercury.

RESULTS AND DISCUSSION

The results of this study have been presented in Table 1. It can be seen that no protection was afforded by prior exposure to reduced L-glutathione or L-cysteine, or by simultaneous exposure to calcium pantothenate, even when a large molar excess of these compounds was used.

Pantethine, when added simultaneously in tank water, apparently protected a large number of fish against mercury toxicity ($\chi^2 = 9.89$, $P < 0.005$ for $HgCl_2$; $\chi^2 = 20.0$, $P < 0.001$ for CH_3HgCl). The result of mercury uptake however, suggests that the protection was due to significantly reduced uptake

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Table 1. Effects of modifiers on accumulation of mercury and survival of goldfish*

Treatment	Mercuric chloride group (400 ng Hg/ml) Hg uptake (ng Hg/g fish) Dead	Alive	Methylmercuric chloride group (80 ng Hg/ml) Hg uptake (ng Hg/g fish) Dead	Alive
Control	8,337 ± 2,386 (9)	10,930 (1)	7,106 ± 1,720 (10)	
L-Glutathione (× 10) 24 hr before	7,008 ± 1,355 (10)		7,569 ± 1,587 (10)	
Calcium pantothenate (10 μg/ml) simultaneously	9,975 ± 2,891 (9)	14,473 (1)	10,069 ± 4,103 (10)	
Pantethine (10 μg/ml) 24 hr before	10,015 ± 4,834 (7)	12,333 ± 2,163 (3)	7,183 ± 862 (9)	9,827 (1)
Pantethine (10 μg/ml) simultaneously	768; 1,138† (2)	3,839 ± 949 (8)		594 ± 368 (10)
L-Cysteine (× 100) 24 hr before	10,658 ± 2,429 (10)		7,318 ±2,540 (10)	
L-Cysteine (× 100) simultaneously		2,715 ± 2,789 (10)		12,136 ± 2,018 (10)
Coenzyme A (0.6 μg/ml) 24 hr before lid placed	13,958 (1)	12,339 ± 1,551 (9)	8,100 ± 931 (10)	
Coenzyme A (0.6 μg/ml) simultaneously lid placed	14,706 ± 5,103 (8)	27,455; 21,351† (2)	5,539; 6,644† (2)	8,780 ± 1,641 (8)

* Values are means ± S.D. Figures in parentheses represent number of fish.
† Individual results of two fish.

of Hg by fish. Possibly the mercury was complexed with pantethine in aquarium water, rendering it unavailable to the fish. When the fish were exposed to mercury 24 hr after exposure to pantethine, more fish survived in comparison to the control group but the difference was not statistically significant (χ^2 was 1.25 and 1.05 for $HgCl_2$ and CH_3HgCl groups, respectively, giving $P > 0.05$).

L-Cysteine, added together with mercurials to the aquarium water, remarkably protected all the fish (χ^2 was 16.36 and 20.0 for $HgCl_2$ and CH_3HgCl groups respectively; $P < 0.001$). An examination of mercury values in fish in the mercuric chloride group revealed that considerably less mercury had accumulated, presumably owing to combination with cysteine in aquarium water. This may be expected in view of the high stability constant of cysteine with mercury [6]. The protection afforded by L-cysteine against methylmercuric chloride toxicity was remarkable not only because of its totality, but also because significantly more mercury was present in these fish than in the fish of the control group ($t = 6.36$; $P < 0.001$). Possibly the simultaneous uptake of cysteine by the fish provided additional high affinity binding sites for mercury which spared "vital sites" from being attacked. Alternatively, the protective effect could have been due to *de novo* synthesis of coenzyme A, a process stimulated by cysteine [7, 8]. In this connection it is pertinent to mention that Stillings *et al.* [9] found that cystine and protein, especially fish protein, reduced the toxicity of methylmercury to rats. The effect of protein, in fact, might have been due to the sulfur-containing amino acids—cysteine and cystine. Similarly, the protective effect of tuna, observed by some workers [10–12], might have actually been due to the higher content of protein and cystine/cysteine in tuna fish [13], rather than to selenium. This contention is supported by our earlier study in which we failed to demonstrate a protective effect of sodium selenite and selenomethionine against the toxic action of mercuric and methylmercuric chlorides on goldfish [5].

The results with coenzyme A were interesting. CoA when added simultaneously with the mercurial afforded significant protection against methylmercuric chloride ($\chi^2 = 13.33$, $P < 0.001$; $t = 2.24$; $P < 0.05$) and not mercuric chloride. Conversely, exposure to coenzyme A, 24 hr before exposing fish to mercurials, protected them against mercuric chloride ($\chi^2 = 12.80$, $P < 0.001$), but not against methylmercury. These are not contrary observations because the lipid soluble and non-ionized substances (methylmercuric chloride and coenzyme A) and ionic compounds (mercuric chloride) accumulate in fish through different pathways [14–16]. Therefore, coenzyme A, penetrating the cell through the same route at the same time as methylmercury, had a greater opportunity to modify mercury toxicity to

the uptake tissues. On the other hand, mercuric chloride, accumulating through a different route than that of coenzyme A, required an initial built-in complement of CoA for protection. This suggests that mercury toxicity may be at least partly due to interference with coenzyme A functions.

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