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Short-term Effects of Two Silver Salts on Tissue Respiration and Enzyme Activity in the Cunner (*Tautogolabrus adspersus*)

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Of the heavy metals most toxic to marine animals, silver is reported to be the most lethal in the bioassay of certain juvenile bivalves (NELSON et al., 1976), to rank second only to mercury in the bioassay of marine larval forms (WALDICHUK, 1974), and to rank third after mercury and cadmium in the bioassay of many adult fish (NAS, 1973). The cunner (Tautogolabrus adspersus), a coastal fish having considerable resistance to environmental challenge, is particularly sensitive to silver; it survives a 96-h exposure to 48 ppm cadmium (NOAA, 1974), but is killed during a similar exposure period by only 1 ppm silver (THURBERG and COLLIER, 1976).

Relative tolerance to silver, as with other metals, varies widely with species (COLEMAN and CEARLEY, 1974), salinity (OLSON and HARREL, 1973; THURBERG et al., 1973; THURBERG and DAWSON, 1974), and temperature (REHWOLDT et al., 1972; MACLEOD and PESSAH, 1973). Still another factor that determines the nature and degree of metabolic stress caused by a metal is its chemical form, a variable that has received little attention thus far. It seemed relevant, therefore, to investigate the effects of two different silver salts on the cunner (T. adspersus) because of its high sensitivity to this metal.

In the work reported here, we examined some effects of a 96-h exposure of cunners to silver either as the nitrate or as the acetate. Our purpose was to determine whether one salt might be considered more toxic than the other, as measured by a common physiological parameter, gill-tissue respiration, and by enzyme activity in liver and skeletal muscle. We wished also to discover whether these different criteria of metabolic stress would lead to similar conclusions regarding the relative toxicities of these two salts.

MATERIALS AND METHODS

Animal exposure: Cunners were collected from Long Island Sound near Milford, Connecticut, and kept in tanks of flowing, sand-filtered seawater (24 ± 2 o/oo salinity) in the laboratory for 1-2 wk of acclimation. During that period they were fed a mixture of Purina Trout Chow and chopped meats of the surf clam (Spisula solidissima), but were unfed for 4-5 days prior to and during each experimental exposure. The fish, ranging in weight

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from 25.9 to 127.6 g (mean wt = 54.6 g) and in length from 130 to 242 mm (mean length = 158 mm), were randomly selected and placed, four per tank, in six 80-liter, all-glass aquaria containing 60 liters of aerated, sand-filtered seawater (24 ± 2 o/oo salinity) maintained at room temperature (22 ± 2 C). Background level of silver in the seawater was 0.001 ppm. Two tanks received silver as $AgNO_3$ to a calculated 0.5 ppm Ag; two other tanks received silver as $AgC_2H_3O_2$, also to a calculated 0.5 ppm Ag; and a final pair of tanks served as untreated controls. Four experimental series were performed in this manner. At the end of each 96-h exposure period, the fish were removed and tissues excised for testing.

Gill-tissue respiration: Two gills were dissected from each fish, rinsed after any clotted blood was removed by gentle teasing, and placed in a 15-ml Warburg-type flask containing 5 ml of the seawater in which the animal had been exposed. Oxygen-consumption rates were monitored in a Gilson Differential Respirometer at 20 C over a 3- to 5-h period, and calculated as µℓ oxygen consumed per h per g dry wt gill tissue ($\mu\ell O_2$/h/g), corrected to µℓ dry gas at standard temperature and pressure.

Tissue preparation: Sample pools of liver (from two to four fish, depending upon size) and skeletal muscle (white tissue only, uniformly from two fish) were stored frozen at -29 C until testing. The liver samples were homogenized in iced, glass-distilled water (1:9, w/v) and centrifuged at 4 C and 17,000 g for 45 min. The supernates were diluted 1:1.5 with the iced water, v/v, recentrifuged, and finally diluted 1:1 for 2% preparations. Skeletal muscle pools were minced and centrifuged for 40 min at 4 C and 30,000 g. The centrifuged tissue fluid (CTF)[1] was diluted 1:49 with the iced water for 2% preparations. Protein content of the tissue preparations was determined by the biuret method (GORNAL et al., 1949) as modified by LAYNE (1957), using a crystallized bovine serum albumin standard.

Assay procedures: The water used in preparing all solutions was doubly glass-distilled and iced, and solutions of coenzymes were made fresh daily. Reaction rates were measured at 340 nm and 25 C; each assay was based on the oxidation or reduction of a pyridine nucleotide coenzyme, the reduced form of which absorbs strongly at this wavelength.

The enzymes for liver study were aspartate aminotransferase (E.C. 2.6.1.1; AAT), a major transaminase already in use as a metabolic criterion (BIESINGER and CHRISTENSEN, 1972;

[1] Abbreviations used in the report are: CTF, centrifuged tissue fluid; ADP, adenosine diphosphate; PEP, phosphoenolpyruvate; ATP, adenosine triphosphate; NADH, the reduced form of nicotinamide adenine dinucleotide, NAD.

GOULD and KAROLUS, 1974; GOULD et al., 1976), and glucose-6-phosphate dehydrogenase (E.C. 1.1.1.49; G6PdH), a magnesium-linked shunt enzyme useful in detecting sublethal stress induced by cadmium (GOULD, in press). The protocol for AAT was the same as that used in a previous study of metal-exposed cunners (GOULD and KAROLUS, 1974), and the G6PdH protocol has also been described in detail elsewhere (GOULD, in press).

For skeletal muscle, we measured the effect of in vitro cadmium chloride on pyruvate kinase (E.C. 2.7.1.40; PK), a magnesium-dependent enzyme operating at a primary metabolic intersection in the mobilization of energy, and highly sensitive to metal ligands (BOYER, 1962). The coupled assay for PK was based on the method of BÜCHER and PFLEIDERER (1955):

$$\text{ADP} + \text{PEP} \xrightarrow{\text{PK}} \text{pyruvate} + \text{ATP}$$

$$\text{pyruvate} + \text{NADH} \xrightarrow{\text{LDH}} \text{lactate} + \text{NAD}$$

The purpose of the assay with added cadmium ion (4 μM assay concentration) was to discover any difference in binding characteristics of skeletal muscle PK between control and exposed fish. Addition of purified lactic dehydrogenase (rabbit muscle, 80 units) did not increase PK activity, either in the standard assay or in the assay with added cadmium. The 3.0-ml reaction mixture comprised: 1.75 ml triethanolamine-HCl buffer, 0.10 M, pH 7.5; 0.10 ml NADH, 4.5 mM; 0.25 ml $MgCl_2.6H_2O$, 100 mM; 0.50 ml KCl, 0.45 M; 0.10 ml 2% CTF; and, to start the reaction after no further oxidation of NADH could be detected, 0.20 ml of a solution of equal volumes of 6.9 mM ADP and 23.4 mM PEP. For the assays containing added cadmium, buffer volume was 1.65 ml, and 0.10 ml 120 μM $CdCl_2.2\text{-}1/2H_2O$ was pipetted into the cuvettes before addition of substrate.

RESULTS

Oxygen-consumption rates were significantly depressed in gill tissues from cunners exposed to silver (0.5 ppm Ag) either as the nitrate or as the acetate salt, with both salts causing the same degree of depression (Table 1).

On the other hand, the two silver salts provoked different responses in the activity of the two liver enzymes examined (Table 2). AAT activity averaged 226 units in the nitrate-exposed fish and 273 in the acetate-exposed fish; average value in the control fish was 252. The difference between the effects of the two salts was significant ($P<.025$).

Silver nitrate depressed liver G6PdH activity ($P<.01$). The acetate salt, however, induced only a small depression that did not differ significantly from the controls (Table 2).

TABLE 1

Gill-tissue oxygen-consumption rates of cunners (Tautogolabrus adspersus) exposed for 96 h to 0.5 ppm Ag as either $AgNO_3$ or $AgC_2H_3O_2$.

Experimental conditions	Number of fish	Oxygen-consumption rates[a] $\bar{x}$	S.E.	(range)	Level of significance[b]	
Controls	22	810	24	(665-1050)	$P < 0.01$	$P<0.01$
$AgNO_3$	21	637	33	(413- 912)	NS	
$AgC_2H_3O_2$	22	599	22	(410- 777)		

[a] $\mu\ell O_2$/h/g dry weight gill tissue.

[b] "Student's" t-test.

TABLE 2

Enzyme activity in the liver of cunners (Tautogolabrus adspersus) exposed for 96 h to 0.5 ppm Ag as either $AgNO_3$ or $AgC_2H_3O_2$.

Enzyme	Experimental conditions	n[a]	Activity[b] $\bar{x}$	S.E.	(range)	Level of significance[c]	
AAT:							
	Controls	11	252	16	(137-331)	NS	NS
	$AgNO_3$	14	226	13	(162-292)	$P < .025$	
	$AgC_2H_3O_2$	13	273	13	(197-342)		
G6PdH:							
	Controls	11	140	11	(84-205)	$P < .01$	NS
	$AgNO_3$	14	104	6	(56-144)	NS	
	$AgC_2H_3O_2$	13	125	9	(87-181)		

[a] Each sample pool comprised livers from 2 to 4 fish.

[b] Unit of activity is micromoles NADH oxidized (AAT) or NADP reduced (G6PdH)/min/mg prot.

[c] "Student's" t-test.

In skeletal muscle, the level of pyruvate kinase activity did not change in either group of silver-exposed fish. However, in vitro cadmium (assay concentration 4 μM) inhibited PK in the controls by 12.5%, and by half again as much (18.6 and 19.1%) in the silver-exposed fish (Table 3). There was no difference in effect between the two salts; skeletal muscle PK in both exposed groups was clearly more sensitive to cadmium inhibition (nitrate, $P<.01$; acetate, $P<.001$) than in the control group:

TABLE 3

Cadmium inhibition of pyruvate kinase in skeletal muscle of cunners (Tautogolabrus adspersus) after a 96-h exposure to 0.5 ppm Ag as either $AgNO_3$ or $AgC_2H_3O_2$.

Experimental conditions	n	Cd inhibition of PK[a] (%)			Level of significance[b]	
		$\bar{x}$	S.E.	(range)		
Controls	10	12.5	1.2	(6.5-19.2)	P<.01	P<.001
$AgNO_3$	10	19.1	1.6	(10.5-26.2)	NS	
$AgC_2H_3O_2$	10	18.6	1.5	(15.5-24.6)		

[a] $CdCl_2.2\text{-}1/2H_2O$ assay concn = 4 μM.

[b] "Student's" t-test.

DISCUSSION

The silver-induced respiratory depression reported here for cunners has also been observed in mud snails (Nassarius obsoletus) exposed for 72 h to 0.5 ppm Ag as nitrate (MACINNES and THURBERG, 1973). In contrast, silver elevated oxygen-consumption rates in six species of bivalves exposed for 96 h to silver nitrate at concentrations ranging from 0.01 to 1.0 ppm Ag (THURBERG et al., 1974, 1975; NELSON et al., 1976). The observations here offer further proof that the nature of silver's toxic effects varies among animal classes.

The biochemical parameters used to assess the relative toxicities of the two silver salts suggest that, in addition, the metal's effects vary with the nature of the silver compound. The G6P-dH data indicate that the nitrate but not the acetate salt produces metabolic stress, but one might consider the difference in effect one of degree only (Table 2). The observed difference between the two salts' effect upon AAT activity is supported by a similar observation in cadmium-exposed rock crabs (C. irroratus). In that case, the chloride salt proved to be more toxic than the nitrate, as measured by heart AAT activity and by mortalities

(GOULD et al., 1976), as well as by gill-tissue oxygen consumption (DAWSON, pers. commun.)[2]. The chloride salt consistently and very significantly elevated AAT values, whereas the nitrate consistently lowered them, although to a much lesser degree; and the difference between the two salts was always greater than the difference between either salt and the controls.

Not surprisingly, PK activity in silver-exposed cunners did not change in the skeletal muscle, where levels of enzyme activity are resistant to environmental influence. Enzyme reactivity with in vitro metal ligands, however, proved to be a more sensitive gauge of enzyme change induced by in vivo metals than were standard measurements of enzyme activity. Skeletal muscle PK activity in both groups of silver-exposed fish was inhibited by in vitro cadmium ion to a significantly greater degree than in the control fish. This observation reinforces other work with metal-exposed marine animals, in whose tissues enzyme sensitivity to in vitro metal ligands changed significantly (GOULD and KAROLUS, 1974; GOULD et al., 1976; THURBERG et al., in press; GOULD, in press).

Poisoning of enzymes is an important mechanism of heavy-metal toxic action. Silver nitrate, by inhibiting G6PdH in the study reported here, interfered more strongly than silver acetate in pentose shunt activity, consequently slowing the flow of metabolites to the biosynthetic pathways for nucleic acids, lipids, and steroids. On the basis of this effect, the nitrate salt might be said to be more toxic than the acetate. The effects of the two salts upon PK ligand sensitivity, however, were similar, as was also true of gill-tissue oxygen consumption. These apparently divergent observations are not necessarily contradictory; physiological stress may arise from the perturbation of any one or more metabolic pathways, and respiratory disturbance, although it may signal general physiological stress, does not distinguish the nature of such stress. In seeking to understand the mechanism of a metal's toxic action, therefore, it is well to look at several metabolic parameters.

It might reasonably be argued that because silver nitrate is more soluble than the acetate, the differences in their effects upon the liver enzymes may be due to available metal-ion concentration. But if such were the case, the differences would be a matter of magnitude only; and the effects observed in this study differ in character as well, as indicated in the AAT data.

Individual toxic effects, especially relatively small ones, may be overcome in time by metabolic compensation, at differing rates and to different degrees. In sequence to this initial study, therefore, we plan a series of long-term exposures with periodic testing, the better to identify those heavy-metal effects that most seriously disrupt metabolism.

[2] DAWSON, M. A., 1976. NOAA, NMFS, NEFC, Milford Laboratory, Milford, Conn. 06460.

In summary, short-term exposure to 0.5 ppm silver as either nitrate or acetate causes respiratory distress in the cunner. Activities of two liver enzymes indicate that the nitrate salt induces a slightly higher metabolic stress than does the acetate.

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Note: Use of trade names is to facilitate description and does not imply endorsement by the National Marine Fisheries Service, NOAA.

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