LONG-TERM CADMIUM STRESS IN THE CUNNER, *TAUTOGOLABRUS ADSPERSUS*

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ABSTRACT

The cunner, *Tautogolabrus adspersus*, was exposed for 30 and 60 days to 0.05 or 0.10 ppm Cd as cadmium chloride. The mean gill-tissue respiratory rates exhibited by the control fish and those exposed to 0.05 and 0.10 ppm Cd were 972, 736, and 665 μl O_2/h·g dry weight, respectively, after 30 days and 1,036, 702, and 587 μl O_2/h·g, respectively, after 60 days. Changes were also observed in the activities of two liver enzymes, aspartate aminotransferase (depression) and glucose-6-phosphate dehydrogenase (induction). Results are compared with those from other metal-exposure studies with cunners and other teleosts.

In recent years cadmium has become the subject of numerous investigations to determine its toxicity to various marine animals. These studies have progressed from short-term exposures to determine the concentrations that cause death (Eisler 1971; National Oceanic and Atmospheric Administration 1974; Westernhagen and Dethlefsen 1975), to long-term exposure studies to measure physiological change caused by very low levels (parts per billion, ppb) of cadmium (Eisler 1974; Calabrese et al. 1975; Dawson et al. in press; Gould in press; Thurberg et al. in press). Such long-term physiological stress can lower an animal's capacity to adapt to and survive in its natural environment.

In a recent collaborative study, a common coastal fish, the cunner, *Tautogolabrus adspersus*, was exposed to cadmium for 96 h and examined for changes in respiration, osmoregulation, cadmium uptake, histopathology, enzyme chemistry, and immune response (National Oceanic and Atmospheric Administration 1974). In the present study, cunners were exposed to cadmium for up to 60 days so that the effects of both exposure regimes might be compared. Parameters selected for study were gill-tissue oxygen consumption, liver enzyme activity, and cadmium uptake by various tissues.

Respiratory activity, a good indicator of the general condition of a fish, has been related to stress caused by such environmental variables as temperature (MacLeod and Pessah 1973), salinity (Olson and Harrel 1973), and heavy-metal pollutants (Calabrese et al. 1975). Gill-tissue respiration correlates well with whole-animal respiration, particularly the standard or inactive rate of oxygen consumption (Vernberg 1956; Thurberg et al. 1975). Thurberg and Dawson (1974) found that a 96-h exposure to 3 ppm Cd caused a depression in the cunner's rate of gill-tissue oxygen consumption. The present study examines the oxygen-consumption rates in excised gill tissue of cunners exposed to lower cadmium concentrations for much longer periods of time.

Because the fish were small, biochemical testing was restricted to the relatively large liver tissue mass. Two enzymes were selected for assay: a key enzyme of nitrogen metabolism that had been tested in the earlier, short-term exposure of cunners to high levels of cadmium (Gould and Karolus 1974), and a magnesium-linked enzyme whose activity in winter flounder, *Pseudopleuronectes americanus*, tissues is affected by the fish's exposure to sublethal levels of cadmium (Gould in press). The first enzyme, aspartate aminotransferase (E.C.3.6.1.1.; AAT), is linked to the production of animal energy (Gould et al. 1976), and in cunners exposed to 24 ppm Cd for 96 h, activity in the liver dropped to 40% of control activity (Gould and Karolus 1974). The second enzyme tested, glucose-6-phosphate dehydrogenase (E.C.1.1.1.49; G6PdH), is the first step in a glycolytic pathway that produces metabolites for reductive biosyntheses, and is found in abnormally high amounts in tissues having the high metabolic rates that often accompany stress (Weber 1963).

Besides the respiratory and enzyme studies,

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chemical analyses were performed to determine the cadmium uptake of certain tissues.

METHODS AND MATERIALS

Cunners for this study were trap-collected in Long Island Sound near Milford, Conn., during the summer of 1974 and held in the laboratory for 1 to 2 wk in flowing, sand-filtered seawater prior to cadmium exposure. They were fed Purina Trout Chow[2] throughout the holding and exposure periods. Beginning in August and ending in October 1974, the cunners were exposed in aerated, 285-liter fiber glass tanks filled to 228 liters with sand-filtered seawater (24±2‰ salinity, 22±2°C) by a proportional-dilution apparatus (Mount and Brungs 1967). This diluter controlled the intermittent delivery of toxicant-containing water to each tank throughout the exposure period at a flow rate of 1.5 liters every 2.5 min. This flow rate provided approximately four complete exchanges of water daily in each tank. Cadmium was added as $CdCl_2 \cdot 2½H_2O$ at concentrations of 0.05 and 0.10 ppm Cd. Background level of cadmium in the seawater was less than 0.001 ppm. Four tanks were used per concentration and control, with 15 fish in each tank, for a total of 60 fish per test level. The fish averaged 55.7 g in weight (range, 32.5-96.9 g) and 157 mm total length (range, 133-185 mm). After 30- and 60-day exposure periods, fish were removed for testing.

For oxygen-consumption measurements, two gills were dissected from each fish and placed in a 15-ml Warburg-type flask containing 5 ml water from the corresponding experimental tank. Oxygen consumption was monitored over a 4-h period at 20°C in a Gilson Differential Respirometer with a shaking speed of 80 cycles/min. Rates of oxygen uptake were calculated as microliters of oxygen consumed per hour per gram dry weight gill tissue ($\mu l\ O_2/h \cdot g$), including the gill arch, corrected to microliters of dry gas at standard temperature and pressure.

Liver tissue was taken for enzyme testing. Pools comprising liver samples from two fish were placed in small plastic pouches from which air was subsequently excluded, then sealed and stored frozen at −29°C. No more than 2 wk elapsed between the end of the exposure period and testing, as both AAT and G6PdH have been found to lose some activity after a month's frozen storage of whole liver tissue. For testing, each liver sample was homogenized 1:9, wt/vol, with iced, doubly glass-distilled water in a small, conical-tip glass homogenizer containing 25-μm glass powder to facilitate grinding. Centrifugation was at 17,000 g and 4°C for 45 min. The supernatant fractions were removed with Pasteur pipettes, diluted 1:1.5 with the iced water, vol/vol, and recentrifuged under the same conditions. The resulting supernates served as the 4% liver preparations. Protein determinations were made by the biuret method (Gornal et al. 1949), with modifications by Layne (1957), using a crystallized bovine serum albumin standard. The coupled spectrophotometric assay for AAT was the same as that used in the acute, short-term exposure of cunners to cadmium described by Gould and Karolus (1974). For G6PdH, both assay medium and spectrophotometric procedures have also been described elsewhere (Gould in press). Unit of activity was micromoles NADH oxidized (AAT) or NADP reduced (G6PdH) per minute per milligram protein.

Gill, muscle, and liver tissues were analyzed for cadmium uptake using the method described by Greig et al. (1975), in which the samples were wet-ashed with concentrated HNO_3, taken up in 10% HNO_3, and analyzed directly by atomic absorption spectrophotometry. Values were calculated on a wet-weight basis.

RESULTS AND DISCUSSION

Mortality and Respiration

Table 1 shows the actual and adjusted mortality data after 30- and 60-day exposures. Mortality data for the exposed fish were corrected for natural mortality of the controls by using Abbott's formula (Finney 1971), and can be interpreted as wholly attributable to cadmium stress. Clearly, exposure to low levels of cadmium increased the incidence of mortality, more so at 0.1 ppm than at 0.05 ppm.

TABLE 1.—Actual and adjusted percent mortality of cadmium-exposed cunner, *Tautogolabrus adspersus*.

Exposure concentration (ppm Cd)	Mortality (%)			
	30 days		60 days	
	Actual	Adjusted[1]	Actual	Adjusted[1]
0.00	3.3 (2)[2]	—	7.5 (5)	—
0.05	10.0 (6)	6.9	18.3 (10)	11.7
0.10	15.0 (9)	12.1	37.4 (20)	32.3

[1]Adjustments made by Abbott's formula (Finney 1971).
[2]Number dead out of 60 fish.

[2]Reference to trade names does not imply endorsement by the National Marine Fisheries Service, NOAA.

TABLE 2.—Gill-tissue oxygen consumption rates of cadmium-exposed cunner, *Tautogolabrus adspersus*.

Exposure concentration (ppm Cd)	Number of fish	Oxygen consumption rates[1] $\bar{X}$	SE	Range	Level of significance[2]	
30 days:						
0.00	10	972	101	754-1,436	$P<0.05$	$P<0.025$
0.05	10	736	46	530- 926	NS	
0.10	12	665	57	420- 967		
60 days:						
0.00	5	1,036	94	788-1,324	$P<0.01$	$P<0.005$
0.05	5	702	37	612- 831	NS	
0.10	5	587	62	472- 810		

[1]Microliters O_2 per hour per gram dry weight.
[2]Student's *t*-test.

Gill-tissue oxygen consumption was significantly reduced after both 30- and 60-day exposures to 0.05 and 0.10 ppm Cd (Table 2), a result similar to that reported by Thurberg and Dawson (1974) in cunners exposed to 3 ppm Cd for 96 h. The depression was more pronounced at the end of the 60-day than at the end of the 30-day exposure. In another chronic exposure study, Dawson et al. (in press) found that gills of juvenile striped bass, *Morone saxatilis*, exposed to 0.5, 2.5, or 5.0 ppb Cd for 30 and 90 days, consumed significantly less oxygen than did the controls. The concentrations used were less than one-tenth of those used in the present study, but they still produced significant respiratory changes. The results reported here are also supported by a study using the winter flounder (Calabrese et al. 1975), in which fish exposed to 5 or 10 ppb Cd for 60 days showed significantly reduced oxygen consumption rates.

Exposure to silver also depresses cunner gill-tissue respiration (Thurberg and Collier in press). There is some evidence, however, that other metals affect fish respiration differently. Cunners exposed to 5 or 10 ppb mercury (as $HgCl_2$) for 30 and 60 days had significantly elevated respiration rates after 30 days, but normal respiration after 60 days (unpubl. data). Similarly opposite effects of the two metals, mercury and cadmium, were reported for the winter flounder in 60-day exposure studies (Calabrese et al. 1975); i.e., mercury elevated the oxygen consumption rate, whereas cadmium lowered it.

Enzyme Activity

In the liver of cunners exposed for 30 days to 0.1 ppm cadmium as chloride, AAT activity was significantly lower ($P<0.02$) than in control fish (Table 3). The drop in activity, about 20%, corroborates the effect of cadmium on liver AAT observed in cunners exposed for 4 days to high concentrations (24 ppm Cd) of this metal salt (Gould and Karolus 1974). As is the case with all aminotransferases, pyridoxal phosphate is an absolute requirement for activity. Because the biosynthesis of this essential cofactor requires a divalent metal cation (Meister 1955), and because cadmium affects enzymes requiring or reacting with divalent metal cations (Gould in press), it seems probable that cadmium's inhibitory effect on AAT activity is at the point of pyridoxal phosphate synthesis.

Liver G6PdH in cunners exposed for 30 days to 0.05 ppm Cd was significantly higher ($P<0.05$) than in controls (Table 3), and at 0.1 ppm the

TABLE 3.—Aspartate aminotransferase and glucose-6-phosphate dehydrogenase in the liver of cunner, *Tautogolabrus adspersus*, exposed for 30 days to cadmium chloride.

Exposure concentration (ppm Cd)	No. of sample pool's	Enzyme activity[1] $\bar{X}$	SE	Range	Level of significance[2]	
AAT:						
0.00	6	233	12	194-281	$P<0.02$	
0.05	6	217	14	160-254		
0.10	6	181	13	154-234		
G6PdH:						
0.00	6	75	11	54- 91	$P<0.05$	$P<0.001$
0.05	6	123	22	78-149	$P<0.01$	
0.10	6	169	12	148-224		

[1]Unit of activity = micromoles NADH oxidized (AAT) or NADP reduced (G6PdH) per minute per milligram protein.
[2]Student's *t*-test.

increase was very highly significant ($P<0.001$). This observation points to elevated pentose shunt activity in the livers of exposed fish. We construe this to be a compensatory mechanism, providing metabolites for increased rates of biosyntheses, to enable impaired biochemical systems to maintain near-normal function. Similar inductive response after sublethal metal challenge has been observed in other teleosts, such as the winter flounder: elevated levels of two metalloenzymes in the kidney and hematopoietic tissue after 60 days' exposure to 0.01 ppm Cd (Gould in press), and elevated levels of ornithine decarboxylase, another pyridoxal phosphate enzyme, in the liver and kidney after intravenous injection of methylmercury, following an initial drop in activity (Manen et al.[3]).

Chemical Uptake

Gill, muscle, and liver tissues from each exposure group were analyzed for cadmium uptake. In contrast to the marked cadmium uptake in tissues of cunners exposed for 96 h to cadmium at levels up to 48 ppm (Greig et al. 1974), nearly all the samples from these 30- and 60-day exposures to both 0.05 and 0.1 ppm Cd, as well as controls, were below the limits of detection (ca. 2 ppm, wet wt) for the sample size and procedure used.

CONCLUSIONS

In summary, long-term exposures of the cunner to 0.1 ppm Cd caused increased mortality, depressed gill-tissue oxygen consumption, and lowered transaminase and elevated pentose shunt activity in the liver.

The toxicity of cadmium to marine animals is influenced, however, by such environmental variables as temperature, salinity, pH, dissolved oxygen (Gardner and Yevich 1969; Vernberg and Vernberg 1972), and chemical form (Gould et al. 1976). Moreover, toxicity of cadmium varies with different species: Westernhagen et al. (1974) and Westernhagen et al. (1975) found that low salinities enhance the toxicity of cadmium to the developing eggs of herring, *Clupea harengus*, and needlefish, *Belone belone*, but Westernhagen and Dethlefsen (1975) reported no such enhancement using flounder, *Pleuronectes flesus*, eggs, possibly because of the differences in the capacity of the egg membranes to bind cadmium ions. The nature and degree of cadmium's toxicity may well change under different laboratory or field conditions.

[3]Manen, C. A., B. Schmidt-Nielsen, and D. H. Russell. 1976. Alterations of polyamine synthesis in liver and kidney of winter flounder in response to methylmercury. Unpubl. manuscr. Univ. Ariz. Med. Cent., Dep. Pharmacol., Tucson, and The Mt. Desert Island Mar. Biol. Lab., Salsbury Cove, Maine.

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