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## EFFECT OF ZINC ON FIN REGENERATION IN THE MUMMICHOG, *FUNDULUS HETEROCLITUS*, AND ITS INTERACTION WITH METHYLMERCURY

Methylmercury has been found to retard fin regeneration in the marsh killifish, *Fundulus confluentus*, and striped mullet, *Mugil cephalus* (Weis and Weis 1978). In *F. confluentus* the retarding effect of methylmercury was masked in water of reduced salinity (9‰). Cadmium, which also retarded fin regeneration in killifish (Weis and Weis 1976), interacted antagonistically with methylmercury so that fish exposed simultaneously to the two metals exhibited growth rates comparable to controls (Weis and Weis 1978).

This paper reports on the effects of zinc on regeneration in the mummichog, *F. heteroclitus*, and the effects of combinations of methylmercury and zinc on this process.

### Methods

Fish were collected by seining in the vicinity of Montauk, N.Y. The lower portion of each caudal fin was amputated with a scalpel, and approximately 15 fish were placed in each of several all-glass aquaria with 10 l of 30‰ salinity water. The temperature was 20°-22° C and the photoperiod was 14 h light/10 h darkness. Fish were fed commerical fish food and live grass shrimp, *Palaemonetes pugio*. Tanks were dosed with methylmercuric chloride (I.C.N. Pharmaceuticals, Plainview, N.Y.[1]) from a 0.1 mg/ml stock solution in 0.2% $NaHCO_3$ to yield a final calculated concentration of 0.050 or 0.025 ppm depending on the experiment, and/or with $ZnCl_2$ (Reagent Grade, Fisher Scientific) from a 1.0 mg/ml stock solution to yield calculated concentrations of 1.0, 3.0, or 10.0 ppm. Aquaria were washed, refilled, and redosed after 2, 4, 7, 9, and 11 days. Regenerating fins were measured with a calibrated ocular micrometer in a stereomicroscope at 7, 9, 11, and 14 days. Experiments were terminated at 2 wk because after that time it became difficult to ascertain the point at which the amputation had been made. The amputation plane can be seen clearly in Figure 1, a control fin 1 wk after amputation.

Three experiments were performed. Experiment I involved exposure of fish 3.5-4.2 cm stan-

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

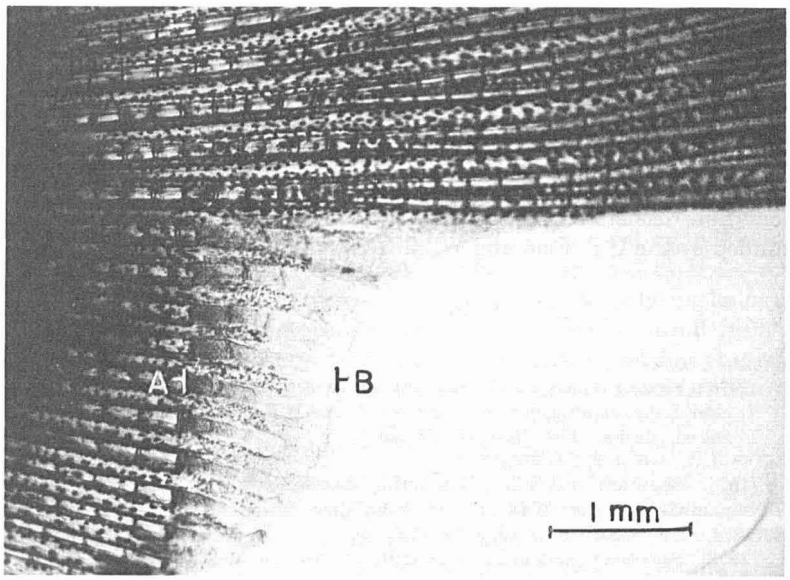


FIGURE 1.—Photograph of regenerating caudal fin, 1 wk after amputation. Measurements were made at A to B.

dard length (SL) to 0.05 ppm methylmercury, 1.0, 3.0, or 10.0 ppm zinc or combinations of 0.05 ppm methylmercury with 1.0, 3.0, or 10.0 ppm zinc. Experiment II was similar, but used 0.025 ppm methylmercury and fish 4.1-5.2 cm SL. Experiment III used 0.05 ppm methylmercury and the same concentrations of zinc, but was performed in water of reduced salinity (10‰) on fish 4.3-5.1 cm SL.

Fish were frozen at the end of some experiments and later analyzed for metal uptake by atomic absorption spectrophotometry (cold vapor technique for mercury, flameless atomic absorption spectrophotometry for zinc). These analyses can be considered accurate within 10%.

## Results

In Experiment I, caudal fin regeneration was retarded by methylmercury and was accelerated by zinc in a dose-dependent fashion. The retardation produced by the mercury could be partially counteracted by the zinc (Figure 2). Analysis of variance of day 14 (Table 1) showed significant effects of mercury, and of zinc, but not of interaction.

Experiment II, using 0.025 ppm methylmercury, produced similar results (Table 2). It can be seen that zinc again accelerated growth in a dose-dependent manner and counteracted the methylmercury-caused depression of growth. Only the group in methylmercury alone and the group in 10 ppm zinc were significantly different from controls ($P \leq 0.05$) as determined by the $t$-test.

TABLE 1.—Analysis of variance on effects of methylmercury and zinc on fin regeneration in *Fundulus heteroclitus*.

| Source of variation | df | SS | MS | $F$ | $P$ |
|---|---|---|---|---|---|
| Hg | 1 | 24.778 | 24.778 | 173.671 | 0.001 |
| Zn | 3 | 2.200 | 0.733 | 5.139 | 0.003 |
| Hg × Zn | 3 | 0.411 | 0.137 | 0.960 | 0.416 |

TABLE 2.—Growth of tail regenerates in *Fundulus heteroclitus* exposed to methylmercury and zinc for 14 days in Experiment II and 11 days in Experiment III.

| | Experiment II | | Experiment III | |
|---|---|---|---|---|
| Exposure | $n$ | mm ±SE | $n$ | mm ±SE |
| Controls | 12 | 3.62±0.078 | 12 | 2.28±0.069 |
| meHg | 15 | 3.31±0.128* | 8 | 1.67±0.125* |
| meHg + 1 ppm Zn | 12 | 3.59±0.099 | 7 | 1.82±0.094* |
| meHg + 3 ppm Zn | 13 | 3.49±0.116 | 2 | 2.14±0.060 |
| meHg + 10 ppm Zn | 12 | 3.50±0.084 | 1 | 2.24±0.00 |
| 1 ppm Zn | 13 | 3.73±0.144 | 11 | 2.43±0.098 |
| 3 ppm Zn | 14 | 3.86±0.142 | 9 | 2.44±0.111 |
| 10 ppm Zn | 10 | 4.18±0.159* | 2 | 2.46±0.020* |

*Significantly different from controls ($P<0.05$) by $t$-test.

In Experiment III (10‰ salinity) a similar pattern was seen (Table 2). High mortality due to interruption in air supply caused the experiment to be terminated early. The groups in methylmercury, methylmercury + 1 ppm zinc, and in 10 ppm zinc were significantly different from controls, as seen by the $t$-test.

Analysis of mercury uptake revealed considerable variation (Table 3). However, it seems likely that the tissue residues are dose dependent and that zinc does not change the uptake of mercury

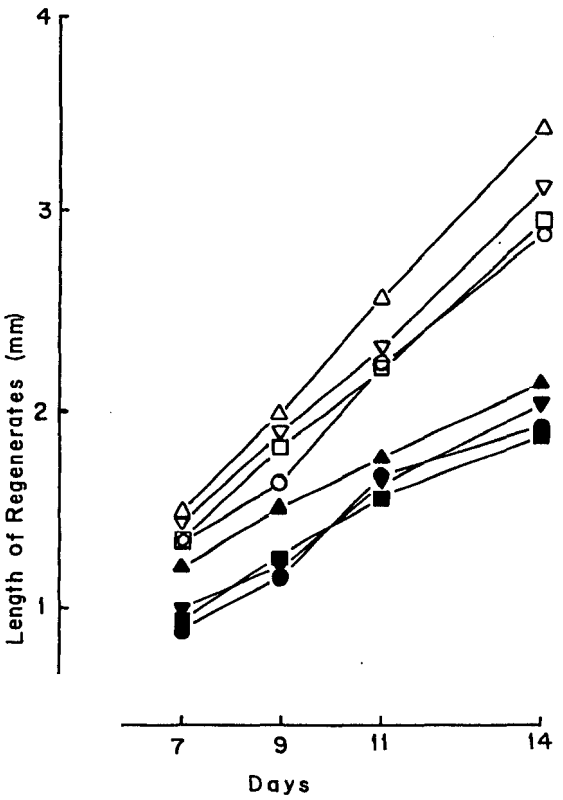


FIGURE 2.—Regenerative growth of tail fin of *Fundulus heteroclitus* exposed to methylmercury and zinc in seawater, Experiment I.
Key: △ 10.0 ppm Zn ($n$ = 15), ▽ 3.0 ppm Zn ($n$ = 15), □ 1.0 ppm Zn ($n$ = 15), ○ Control ($n$ = 14), ▲ 0.05 ppm meHg + 10.0 ppm Zn ($n$ = 11), ▼ 0.05 ppm meHg + 3.0 ppm Zn ($n$ = 12), ■ 0.05 ppm meHg + 1.0 ppm Zn ($n$ = 9), ● 0.05 ppm meHg ($n$ = 12).

TABLE 3.—Average mercury uptake (ppm Hg/wet weight ± SE) by *Fundulus heteroclitus*.

| Item | Control | | Hg | | Hg + 10 ppm Zn | |
|---|---|---|---|---|---|---|
| | Uptake | $n$ | Uptake | $n$ | Uptake | $n$ |
| Experiment I: | | | | | | |
| Carcass | n.d.[1] | 3 | 32±5.0 | 3 | 37±7.2 | 3 |
| Brain | n.d. | 3 | 11±0.9 | 3 | 19±4.8 | 4 |
| Experiment II: | | | | | | |
| Carcass | n.d. | 3 | 7.4±0.7 | 3 | 15.4±3.1 | 5 |
| Brain | n.d. | 3 | 4.8±2.6 | 3 | 9.7±1.6 | 5 |
| Experiment III: | | | | | | |
| Carcass | n.d. | 3 | 33±12 | 3 | 25±1.4 | 3 |
| Brain | n.d. | 3 | 28±6.0 | 3 | 25±2.5 | 3 |

[1]n.d. = not detectable, <0.03 ppm.

into the brain or the rest of the body. Accumulation of zinc was not altered by methylmercury. Animals in 10 ppm Zn accumulated 246±1.41 ppm; those in 10 ppm Zn + 0.05 ppm meHg accumulated 250±3.54 ppm. Those in 1 and 3 ppm Zn accumulated 221±25.2 and 250±4.95 ppm, showing no clear dose-dependent relationship.

## Discussion

The data indicate that in *F. heteroclitus*, zinc can accelerate regenerative growth, and, by so doing, can counteract the retarding effects of methylmercury. In this species, the regeneration rate of controls was similar in 30‰ and 10‰ salinity, and the methylmercury retarded growth at both salinities. This is in contrast to *F. confluentus* in which decreased salinities depressed the regeneration rate, thus masking the effects of methylmercury in water of 9‰ salinity (Weis and Weis 1978).

Methylmercury has previously been observed to retard regeneration (Chang et al. 1976; Weis and Weis 1978) and other developmental processes (Chang et al. 1974). Its action as an inhibitor of mitosis (Ramel 1969) could be the cause of these effects on growth processes. As a potent nerve poison it could further inhibit regenerative growth by interfering with the neurotrophic influence necessary for regeneration.

Previous studies on the effects of zinc on growth include the work of Hirsch and Hurley (1978) in which zinc was found to counteract the teratogenic effects of 6-mercaptopurine in the rat. They felt that the drug lowered DNA synthesis and that the zinc counteracted this. Swenerton et al. (1969) correlated zinc deficiency with reduced DNA synthesis in rat embryos, and Falchuck et al. (1975) have associated zinc with promoting cell division in *Euglena gracilis*. Thus, if zinc can promote DNA synthesis and cell division in fish also, that would account for the observed acceleration of regenerative growth. However, previous studies on fish have not indicated such an effect. Crandall and Goodnight (1962) reported that 1.15 ppm zinc retarded the growth of newborn guppies. Rachlin and Perlmutter (1969) found that 18 ppm Zn reduced the mitotic index of cultured rainbow trout, *Salmo gairdneri*, cells, but that 1.8-10.0 ppm had no effect on the mitotic index.

On the other hand, zinc has often been found to counteract toxic effects of other heavy metals. Dixon and Compher (1977) found that zinc could reverse a cadmium-caused inhibition of regeneration in the newt. Zinc has been found to counteract the toxic effects of mercury in rats (Yamane et al. 1977) and to counteract the teratological effects of methylmercury in killifish embryos (Weis et al. in press).

In view of reports of fin rot of unknown etiology in flatfish from polluted environments (Ziskowski

and Murchelano 1975), the retardation of growth by heavy metals may be of significance in inhibiting regeneration of fins eroded by the benthic substrate.

## Addendum

It has recently been demonstrated that, in certain poeciliid fishes, some environmental variables which affect general growth rate do not affect the rate of fin regeneration. Factors which do cause differences in length of regenerated fin generally affect the time needed for wound healing and blastema formation, rather than rates of regeneration per se (E. Zimmerer, Ph.D. dissertation, Rutgers University, 1980). We tested the data represented in Figure 1 for this possibility. In regression analysis, the slope equals regenerative rate per se and the elevation ($y$-intercept) represents the time needed for wound healing and blastema formation. Analysis of covariance indicates that when Hg treated fish are compared with control fish, both the slopes and $y$-intercepts are significantly different ($F$ = 10.23 and 80.76, respectively). Similarly, when 10 ppm Zn treated and control fish are compared, the slopes and $y$-intercepts are significantly different ($F$ = 6.83 and 41.29, respectively). Therefore, it appears that these heavy metals affect both the initial wound healing and blastema formation and the rate of regeneration per se.

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