Effects of Heavy Metals on Fin Regeneration in the Killifish, *Fundulus heteroclitus*

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After amputation, teleost fins can regenerate by forming a blastema at the cut ends of the fin rays (lepidotrichia) (GOSS and STAGG, 1957). After the initial organization of the blastema, new lepidotrichia form, which become attached to the ends remaining in the stump (KEMP and PARK, 1970). The rate of regeneration is proportional to the amount removed (TASSAVA and GOSS, 1966) and can be affected by the age and size of the fish (COMFORT and DOLJANSKI, 1958) and the quality of the water in which it lives. The insecticides DDT, malathion and Sevin were found to retard the rate of fin regeneration in the killifish, Fundulus heteroclitus (WEIS and WEIS, 1975).

Heavy metal pollution of the marine environment, a subject of considerable concern, generally results from industrial waste discharge. These chemicals tend to accumulate in organisms and have been found to have a variety of adverse effects on fishes. There have been numerous direct toxicity studies (BALL,1967; EATON, 1974;EISLER, 1971; PICKERING and HENDERSON, 1966) as well as studies of biochemical effects (ABOU-DONIA and MENZEL, 1967; GOULD and KAROLUS 1974; JACKIM et al, 1970) and physiological effects (BIOKO, 1965; GARDNER and YEVICH, 1970; ROBOHM and NITKOWSKI, 1974; THURBERG and DAWSON, 1974). The studies of CRANDALL and GOODNIGHT (1962) and DORFMAN and WHITWORTH (1969) have indicated that heavy metals can retard growth in fishes.

The following experiments were designed to study the effects of mercury, lead and cadmium on fin regeneration in Fundulus.

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MATERIALS AND METHODS

Fundulus heteroclitus, 4-5 cm in standard length, were collected from Lake Montauk, N.Y., by seining. The lower half of the caudal fin was amputated one mm from its base with a sharp scalpel. The fish were maintained in glass jars containing 6 fish in a volume of four liters of sea water, with slow aeration, at ambient temperature (25 C) and 30% salinity. The fish were fed commercial fish food frequently. Experimental fish were treated with Pb, Hg or Cd dissolved in the water.* The jars were washed and redosed twice weekly. [In a similar experimental design, JACKIM et al (1970) determined that the loss of metal ion from solution over a 96-h period was 0% for Cd, 26% for Hg, and 79% for Pb.] After one, one and one-half, and two weeks, the regenerates were measured by a calibrated ocular micrometer in a stereomicroscope. The lengths of regenerates were analyzed by Student's t-test for independent observations.

RESULTS

In the first experiment, fish were exposed to Hg at 0.1 mg/l and 1 mg/l, Pb at 0.1 mg/ l and 1 mg/l, and Cd at 0.01 mg/l and 0.1 mg/l (Table 1). One mg/l Hg was lethal to the fish and all were dead before the first measurement (as predicted by JACKIM et al, 1970). Those in 0.1 mg/l Hg survived over 1½ weeks and subsequently died. Those in 0.1 mg/l Pb regenerated at slightly faster rates than the control fish, but those in 0.01 mg/l Cd regenerated at a slower rate than did the control fish. Those in the higher concentration of Cd (0.1 mg/l) were likewise slower than the controls but the difference was not always significant. Therefore, the experiment was repeated with three dose levels of Cd and with 12-15 fish per group. The fish were distributed in equivalent amounts of seawater in their jars (Table 1 and Figure 1).

All three doses of Cd strikingly retarded the rate of regeneration, although the effect was not correlated with the dose. At the one-week measurement, many of the Cd-treated fish had tails which appeared to have

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*Pb as $Pb(NO_3)_2$, Hg as $HgCl_2$, both as reagent grade from Fisher Scientific Co.; Cd as $CdCl_2$, reagent grade, Matheson, Coleman and Bell.

TABLE 1

Length of regenerated caudal fins, in mm.

Group	Day 7	Day 10	Day 14
Experiment 1			
Control	2.0 ± 0.1	3.2 ± 0.2	4.1 ± 0.2
Hg 0.1 mg/l	1.7 ± 0.3 (n.s.)	2.6 ± 0.3 (n.s.)	-----
Pb 0.1 mg/l	2.4 ± 0.1 ($p < .05$)	3.6 ± 0.1 (n.s.)	4.7 ± 0.3 (n.s.)
1.0 mg/l	1.7 ± 0.1 (n.s.)	3.3 ± 0.1 (n.s.)	4.0 ± 0.3 (n.s.)
Cd 0.01 mg/l	1.6 ± 0.1 ($p < .05$)	2.6 ± 0.2 (n.s.)	3.0 ± 0.2 ($p < .01$)
0.1 mg/l	1.5 ± 0.2 ($p < .05$)	2.7 ± 0.3 (n.s.)	3.8 ± 0.3 (n.s.)
Experiment 2			
Control	2.2 ± 0.1	4.0 ± 0.1	4.7 ± 0.1
Cd 0.01 mg/l	1.2 ± 0.1 ($p < .001$)	2.9 ± 0.1 ($p < .001$)	3.3 ± 0.1 ($p < .001$)
0.1 "	1.1 ± 0.1 ($p < .001$)	2.4 ± 0.2 ($p < .001$)	3.1 ± 0.3 ($p < .001$)
1.0 "	1.1 ± 0.2 ($p < .001$)	2.8 ± 0.2 ($p < .001$)	3.4 ± 0.2 ($p < .001$)

n.s. = not significant ($p > .05$)

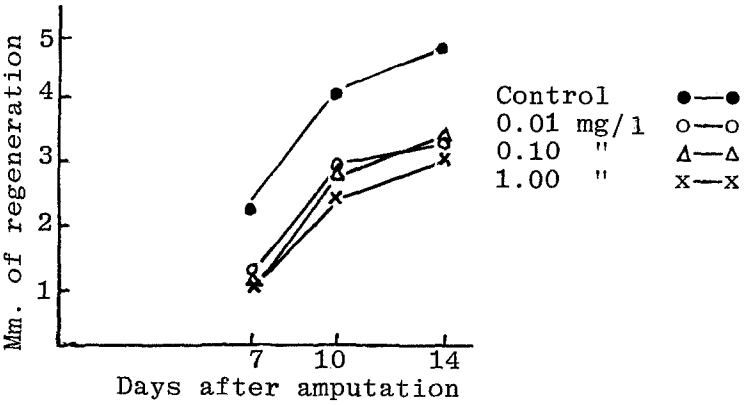

FIG.1. Retardation of fin regeneration by cadmium

stasis of circulation, with much blood visible in the blastema where control tails already had significant amounts of new fin ray visible. This had also been observed in the previous experiment, and probably reflected a severe problem in the healing process. That the effect is on the initial healing and blastema formation rather than on growth per se is indicated by Figure 1 in which the growth rates of experimental and control fish are seen to be about equal; the control fish, having started sooner, stay ahead.

DISCUSSION

Growth in this system was not retarded by lead as were the systems of CRANDALL and GOODNIGHT (1962) and DORFMAN and WHITWORTH (1969). Lead at 0.1 mg/l seemed to have, if anything, an accelerating effect. In this regard, regeneration seems to be affected differently from absolute growth in fish. Mercury was the most toxic to the killifish, but its retardation of regeneration barely reached statistical significance at dose levels which proved lethal to all the fish before the two weeks had elapsed in the experiment. Cadmium, on the other hand, was not toxic to the fish at these dose levels, but did have a retarding effect, especially on the early stages of blastema formation.

THURBERG and DAWSON (1974) found that Cd (3 ppm) reduced oxygen consumption in the cunner (Tautogolabrus adspersus). GOULD and KAROLUS (1974) found decreased liver enzyme activity, also in the cunner. JACKIM et

al (1970) similarly found reduction of liver enzyme activity in Fundulus. Another possible mechanism producing reduced oxygen consumption with Cd was the gill tissue damage described by GARDNER and YEVICH (1970). In either case, an overall lowered respiratory rate and metabolism might have been responsible for the retardation of the regeneration. A slight reduction in growth rate was found in bluegills exposed to Cd (CEARLY and COLEMAN, 1974). The bloody appearance of the blastemas at one week may have been related to the lesions observed by EISLER and GARDNER (1973) in epithelia and kidneys of fish exposed to combinations of metals.

The fact that all three dose levels of Cd produced an equivalent degree of retardation may be attributed to a limit in the amount of Cd that the fish can accumulate. It was found by EISLER (1974) that Cd, unlike Hg, does not build up in the tissues of Fundulus. This can account for the lack of a dose-related effect of fin regeneration.

The absence of fins, or parts of fins, puts a fish at a disadvantage in nature. Thus it is beneficial to regenerate a missing part quickly. A retardation of the regenerative processes, caused by pollutants such as Cd is another detrimental effect of this chemical on marine organisms. If the bloody appearance of the one-week regenerates in Cd reflects an interference with healing, then this would be another even more serious effect of Cd on this fish.

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