Effect of Iron on the Growth Rate of Fishes [1,2]

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ABSTRACT To determine the nutritional requirment for iron in fish growth, the effect of daily addition of supplemental ferrous sulfate on the growth of *Xiphophorus helleri* and *Xiphophorus maculatus* (the swordtail and the platyfish) was measured by determination of weight gain and hematocrit levels. Both variables increased as a result of the added iron. The effectiveness of the treatment diminished as sexual maturity was approached. Treatment with ferrrous sulfate led to an increase in growth rate even in fish fed maximally with live brine shrimp. Ferric salt was not judged effective. Addition of ferrous iron also significantly decreased the mortality from hatching to maturity.

Little is known of the mineral requirements, or of the mineral metabolism of fishes. Lovelace and Podoliak (1) showed that calcium is absorbed through the gill of the brook trout, and Phillips and his co-workers (2) have reported dietary requirements for growth in trout, although their main concern was with the organic constitutents of prepared hatchery feeds. They determined calcium, phosphorus, and magnesium levels in their diets, but did not systematically vary these. Cheprakova (3) reported some effects of iron salts on developing eggs of the loach, the sig, and the perch, but the data reported in that paper are not at all conclusive.

In connection with studies in our laboratory concerned with respiratory rate and other factors in the swordtail (*Xiphophorus helleri*), the platyfish (*Xiphophorus maculatus*) and the hybrid *helleri* × *maculatus* (4), we noted that the addition of ferrous sulfate to water in which the fishes were raised appeared to have a stimulatory effect on the growth of these fishes, as compared with untreated controls. Accordingly studies were made in which we systematically varied the amount and kind of iron available to newly hatched fry and to older fishes, and compared weight changes and changes in hematocrit values of treated to untreated animals.

METHODS

Fish were maintained in 13.5-liter aquariums, filled with water withdrawn from the Wakulla River. The pH of the water was adjusted to between 7 and 8, and Anacharis (Elodea) and calcium carbonate blocks were added to the tanks. Snails were introduced and allowed to grow. As soon as possible after swimming fry appeared in a tank, they were removed, and fry of a single brood were separated into groups of equal size, and placed into newly established aquariums. Fry were fed an average of 90 mg of dry food/day/tank, with a supplement of 300 mg of liquid food, containing 50 mg dry weight.[3] The dried food contained 45% crude protein and 3% crude fat, with a maximal fiber content of 4%, and the analysis of the liquid food showed 5% crude protein, 1% crude fat with a maximal crude fiber content of 1.5%. The iron content of these feeds, of the aquarium water, and of live brine shrimp was measured, using a modification of the technique of Sherman et al. (5), in which alpha-alpha' dipyridyl is used as the color reagent, and which is reported to measure biologically

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[2] We wish to thank Wardley Products, Inc., Long Island City, New York, for their courtesy in supplying dried food diets and numerous other aquarium supplies.
[3] Dried food was prepared from: animal liver meal, whale meal, meat meal, menhaden fish meal, crab meal, shrimp meat, salmon egg meal, wheatgerm meal, dried *Daphnia*, kelp meal, oat meal, wheat flour, corn meal, soya flour, 3% whole egg solids, 3% dried skim milk, 3% salt, 2% mosquito larvae, 2% ground aniseed, 1% calcium triphosphate, 1% barley malt and 1% primary dried yeast. Liquid food supplement contained: whole eggs, yeast hydrolysate, 3% cod liver oil, 2.5% kelp meal, 2% animal liver meal, 0.5% spinach powder, 0.5% gum acacia, 0.5% gum tragacanth, 1% dextrin, water and 0.25% sodium bisulphite.

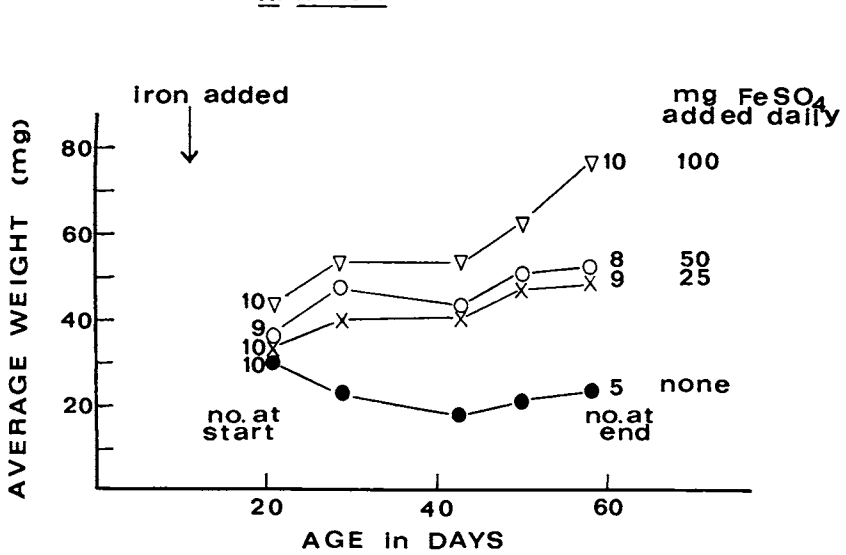

Fig. 1 Effect of varying concentrations of ferrous sulfate on the growth of the green swordtail, *Xiphosphorus helleri*.

available iron, as measured by growth responses, rather than total iron, which may be bound and not available for absorption into the blood of the organism. The aquarium water contained less than one part per million of iron, making the total content of an untreated tank less than 0.2 mg. Daily rations of food contained less than 0.07 mg available iron, while an average feeding of live brine shrimp (*Artemia* sp.) contained only 5×10^{-4} mg. In comparison with the amount of added iron these are considered to be negligible levels. Supplemental iron salts were added daily at the indicated levels, as analyses for soluble iron showed complete disappearance of the dissolved salt after 4 hours. Copious precipitates of ferrous hydroxide were observed in all treated tanks. Hematocrit levels were measured using a modification of the orbital bleeding technique of Riley (6) in heparinized micro-hematocrit tubes.

EXPERIMENTAL RESULTS

Initially, 40 fish from 2 broods of swordtails were combined, and then separated into 4 tanks, each containing 10 fish. Three groups were treated by daily addition of varying amounts of ferrous sulfate, and one was kept as a control group. The results of this initial experiment are shown in figure 1. After 60 days, the differences in weight and in survival were striking, especially as a higher growth rate in the tanks containing fewer fish would be expected (7). After 100 days the groups which had received 50 and 100 mg of ferrous sulfate were divided, and the treatment continued for half, and discontinued in the other half. The data are presented in figure 2, and indicate that continued treatment with the salt loses its effectiveness after 100 days. The apparent depressing effect of the iron treatment on the fish in the group to which 100 mg had been continued may be explained by noting that in this group no evidence of sexual maturity was noted, whereas in the group which had been removed from treatment with 100 mg two of five had already shown marked sexual differentiation. In swordtails, the striking sexual dimorphism leads to both size and weight differences between the sexes, females being larger and heavier than males. When secondary sex differentiation occurs, then, the data can no longer be treated as coming from a common pool, but would have to be paired for sex. The indication that iron treatments lose effectiveness was confirmed by several attempts to change growth rates in adult fish after they had been

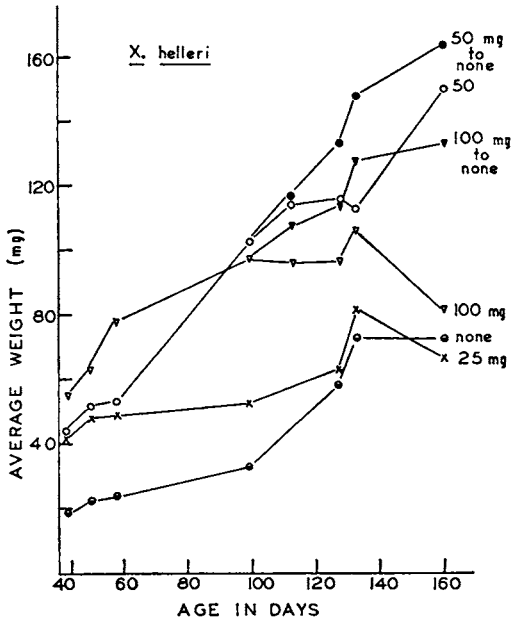

Fig. 2 Decreasing effectiveness of ferrous sulfate treatment after 100 days from hatching. The 50- and 100-mg groups were split at 100 days, and treatment was discontinued in half of each group.

raised with no iron present; no changes in growth rate or in hematocrit values were observed in any of these experiments.

The data from 2 experiments utilizing the platyfish are presented in figure 3. In the lower portion of the figure the curves indicate that after 75 days the growth rate of the control group exceeded that of the 50-mg group, and that the growth rate before this time, as indicated by the slope of the curves, is approximately the same for the control, 25-mg, and 50-mg groups. These data must be treated with caution, as by the 44th day, two of the 25-mg group and three of the control group had died, whereas all of the treated fish still survived, and did so to the end of the experiment. On the upper portion of the figure, 2 groups in which the survival rate was similar are compared. With similar mortalities the growth rate of the treated fish exceeds that of the control group by 65%.

Efforts were made in subsequent experiments to balance any changes which might have resulted from crowding ef-

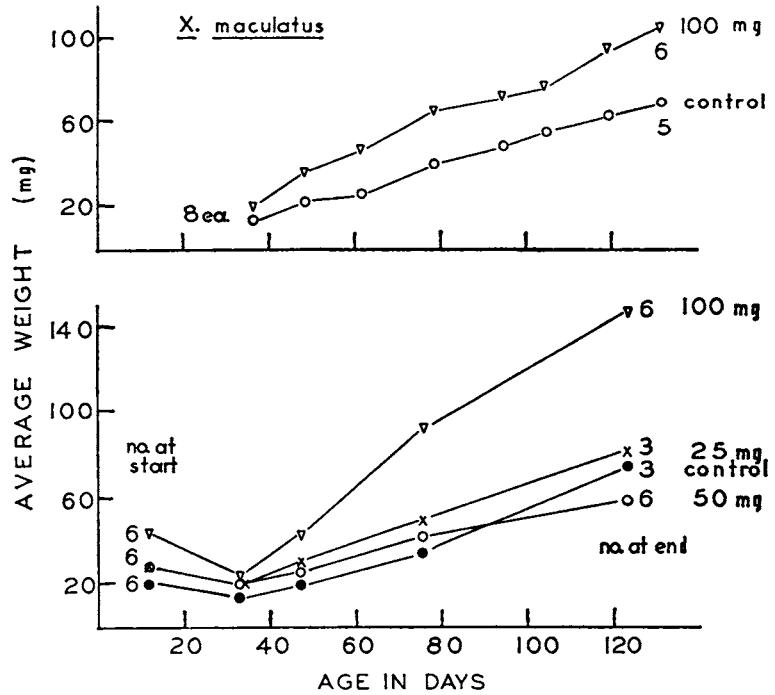

Fig. 3 Effect of daily addition of ferrous sulfate on the growth of the platyfish, *Xiphophorus maculatus*. After the 44th day only 3 control fish were left, and only four in the 25-mg treated group, in the lower portion of the figure.

fects by removing fish from the various groups in which little or no mortality occurred, so that the size of the control and experimental groups remained similar throughout the experiment. Difficulties of this sort led us to examine our data in terms of increased survival due to supplemental iron treatments. These data are presented in table 1, in which all experiments using both platyfish and swordtails are combined. Utilizing a contingency chi-square test the differences in survival rates of the groups treated with 50 mg and 100 mg are significant, P being less than 0.01, whereas the 25-mg groups gave a P value between 0.1 and 0.2. Accordingly the use of the 25-mg supplement was discontinued as being, at best, submaximal.

The addition of live brine shrimp (*Artemia*) is often used to supplement fish feeding regimens, with excellent results reported in terms of fish growth (8). Accordingly a brood of fry was separated into 2 equal groups and one, used as a control, was fed maximally a diet supplemented daily with brine shrimp; the other was fed in the same way but daily additions of 100 mg of ferrous sulfate were also made. Figure 4 shows that until 66 days after hatching a stimulation due to added iron was still present.[4] Both groups grew faster on this regimen than with a diet with only occasional brine shrimp supplements, but it appears that brine shrimp do not supply enough iron, at least in the early growth stages.

Together with increased growth of the treated fish we observed an increase in hematocrit levels, when compared with control groups. In analyzing these data it became necessary to pool the results of many experiments, as the value of the hematocrit, from control group to control

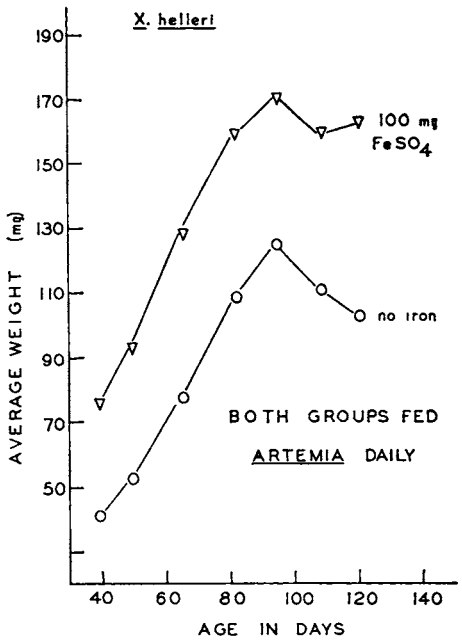

Fig. 4 Effect of the addition of ferrous sulfate to swordtails fed live brine shrimp daily.

group, was highly variable, for reasons not clear. These data are presented in table 2. In no case did the hematocrit value of a control fish exceed the value for any in the treated group matched with that control, although on occasion one control group might have a higher value than that observed in a different experimental group. Despite the overlap in standard deviations, the differences, when subjected to an analysis of variance, are significant at the 5% level of confidence.

Similar experiments were made using ferric nitrate as the iron source, and add-

[4] The ratio of the slope of the treated to the untreated group is: days 40 to 50, 1.7/1.1; days 50 to 66, 2.15/1.56.

TABLE 1

Effect of treatment with ferrous sulfate on survival of platyfish and swordtails from hatching to 120 days

Treatment	Initial no.	Final no.	Mortality
			%
None (control)	59	24	58
25 mg $FeSO_4$ added daily	16	10	38
50 mg $FeSO_4$ added daily	15	14	8
100 mg $FeSO_4$ added daily	59	45	24
Combined treated group	90	69	23

TABLE 2

Hematocrit levels of fishes with various treatments [1]

Type of fish	Daily treatment		
	100 mg $FeSO_4$	50 mg $FeSO_4$	Control
Xiphophorus helleri (green swordtail)	38.0 ± 6.6 [2] (14) [3]	40.0 ± 4.0 (22)	37.2 ± 4.9 (34)
Xiphophorus maculatus (platyfish)	38.7 ± 6.8 (15)		32.1 ± 4.3 (16)
Hybrid (*helleri* × *maculatus*)		40.22 ± 1.4 (8)	31.7 ± 0.04 (5)

[1] We are indebted to Miss Patricia Hopper for the data on the hybrid fishes, and for much of the data on swordtails.
[2] Mean packed cell volume/100 ml ± SD.
[3] Numbers in parentheses indicate number of fish.

ing the salt at the level of 100 mg/day to tanks containing new-hatched swordtail fry. At the end of an 84-day period the average weight and the rate of growth of the control group and the treated fish were essentially the same, 2 groups of treated fish had average weights of 145 mg, and 2 control groups 130 mg, (144 treated vs. 144 control; 146 treated vs. 117 control). We conclude that this treatment does not appear effective in stimulating growth; hematocrit values in both groups were not statistically different (35.5 treated vs. 34.9 control).

DISCUSSION

The observed increases in growth rate and in hematocrit levels indicate that, as with chicks, iron is an essential nutrient for these fish during the period immediately following hatching. As sexual maturity is approached the stimulation due to iron addition to the diet disappears, and it is absent in adult fish. Absorption probably takes place across the gill membrane, and is evidently accomplished rapidly, as the disappearance of soluble iron from solution limits the time of exposure. Since ferric iron does not appear to be utilized, it is possible that the mechanics of transport bear some relationship to the absorption of iron through the intestinal mucosa in mammalian systems (9). The rise in hematocrit levels indicates that the iron is both absorbed and utilized. Further experiments with chelated iron and with possible synergistic effects of other minerals are in progress.

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