GENERAL AND COMPARATIVE ENDOCRINOLOGY 86, 398–406 (1992)

Stimulation of Coho Salmon Growth by Insulin-like Growth Factor I[1]

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Accepted October 7, 1991

The effect of insulin-like growth factor I on growth rate of coho salmon (*Oncorhynchus kisutch*) was examined. Juvenile coho salmon received implants of osmotic minipumps containing recombinant bovine insulin-like growth factor I (rbIGF-I) or saline for a period of 3 to 4 weeks. High doses of rbIGF-I (>0.13 $\mu g \cdot g^{-1} \cdot d^{-1}$) resulted in hypoglycemia and death. In 2-year-old coho salmon, 0.09 $\mu g \cdot g^{-1} \cdot d^{-1}$ rbIGF-I administered for 25 days doubled linear growth rate and increased growth rate in weight by 40%. In rapidly growing, 1-year-old coho salmon, growth rate was not altered by rbIGF-I at 0.01 or 0.05 $\mu g \cdot g^{-1} \cdot d^{-1}$ for 31 days. In ration-limited fish exhibiting slow growth in the control group, rbIGF-I (0.02 to 0.12 $\mu g \cdot g^{-1} \cdot d^{-1}$) increased linear growth rate by up to threefold and growth rate in weight by up to fourfold. The results indicate that exogenous treatment with mammalian IGF-I can stimulate coho salmon growth under some conditions, and that endogenous IGF-I may be an important factor in regulating growth of teleosts.

The importance of growth hormone (GH) in the regulation of growth is implied by its name and has been verified in a wide variety of vertebrates (e.g., Donaldson *et al.*, 1979; Weatherley and Gill, 1990). GH stimulation of insulin-like growth factor I (IGF-I) production by hepatic and extrahepatic tissues and stimulation of growth by IGF-I have been widely demonstrated in mammals (see Daughaday and Rotwein, 1989). Some debate revolves around the relative importance of GH and IGF-I in stimulating growth and differentiation of target tissues (Isaksson *et al.*, 1987; Skottner *et al.*, 1987; Daughaday and Rotwein, 1989). Given the known growth-promoting effects of GH in teleost fishes, an understanding of the action of IGF-I on whole animal growth rates can help clarify the relative roles of GH and IGF-I in growth regulation.

The recent availability of recombinant growth factors has made *in vivo* studies of IGF-I feasible. In mammals, IGF-I infusion can increase growth rate in hypophysectomized (Schoenle *et al.*, 1982; Skottner *et al.*, 1987; Guler *et al.*, 1988, 1989), diabetic (Scheiwiller *et al.*, 1986), and intact (Hizuka *et al.*, 1986) rats, and in dwarf mice (van Bull-Offers *et al.*, 1986). Guler *et al.* (1989) reported no effect of IGF-I infusion in miniature poodles, using methods that were successful in hypophysectomized rats.

There are few studies on the effects of IGF-I on growth of nonmammalian vertebrates. Skyrud *et al.* (1989) reported that weekly bolus injection of human IGF-I in brook trout (*Salvelinus fontinalis*) had no effect on growth at low doses and de-

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0016-6480/92 $4.00

creased growth rate at high doses. These injections also caused a dramatic hypoglycemia (Skyrud *et al.*, 1989), suggesting that IGF-I was interacting with the insulin receptor (Froesch and Zapf, 1985). The present study investigates the effects of constant infusion of mammalian (recombinant bovine) IGF-I on the growth of a teleost, the coho salmon (*Oncorhynchus kisutch*).

MATERIALS AND METHODS

Hormone administration. Recombinant bovine insulin-like growth factor I (rbIGF-I) was obtained from the Monsanto Corp. (St. Louis, MO). The content of rbIGF-I was greater than 95% as determined by high pressure liquid chromatography, and contained no detectable IGF-II. The hormone was dissolved in sterilized 0.9% NaCl. Hormone solution and saline vehicle were placed in osmotic minipumps (Alzet, Palo Alto, CA) just prior to implantation. Each pump was labeled with indelible ink to permit identification of individual fish at the end of the experiment. Fish were starved for at least 30 h prior to implantation. Following anesthesia (0.4 ml/liter 2-phenoxyethanol), an incision was made in the lower lateral musculature (just anterior and dorsal to the pelvic fin) and the minipump inserted into the peritoneal cavity. The wound was covered with an antibiotic salve and closed with a single stitch. A prophylactic antibiotic (Maracyn) was added to the water for a 24-hr period following implantation. Fish resumed feeding within 1 day of the implant procedure. Effective dose was calculated from the initial weight of the fish and the manufacturer's specified pumping rate for the temperature of each experiment. Since the weight-specific dose will vary as the weight of fish in each group varies, doses are expressed as an average (based on the average initial weight of the fish in a group), and range (in brackets).

Animals. Coho salmon (*O. kisutch*) obtained from Iron Gate Hatchery (California Department of Fish and Game) were reared in freshwater raceways at the Bodega Marine Laboratory. At least 2 weeks prior to an experiment, fish were transferred to 1-m diameter, 800-liter capacity tanks at the University of California at Berkeley. A recirculating biofiltration unit supplied continuous water flow. Fifty percent of the water was replaced every other day. Fish were fed Oregon Moist pellet (Moore–Clarke, LaConner, WA) *ad libitum* twice daily.

Experiment 1. One-year-old juvenile coho salmon with initial weights of 40–67 g were randomly separated into two groups. On July 7, each group received implants of osmotic minipumps (as previously described), those of one group containing 3.5 mg/ml rbIGF-I (0.41 $\mu g \cdot g^{-1} \cdot d^{-1}$) and the others saline vehicle. Temperature was initially 15 ± 1°, but after mortalities occurred in the rbIGF-I group (3 days after implantation), it was lowered to 8 ± 1° to reduce the effective dose. Fish were held in the Life Sciences Building courtyard (natural photoperiod) and fed *ad libitum* twice daily.

Experiment 2. Two-year-old juvenile coho salmon with initial weights of 104–272 g were randomly separated into two groups. On July 17, each group received implants of osmotic minipumps, those of one group containing 3.5 mg/ml rbIGF-I (0.13 $\mu g \cdot g^{-1} \cdot d^{-1}$) and the others saline vehicle. Temperature was maintained at 10 ± 1° throughout the experiment. Fish were held in the Life Sciences Building courtyard and fed *ad libitum* once daily.

Experiment 3. One-year-old juvenile coho salmon with initial weights of 66–108 g were randomly separated into three groups. On October 10, each group received implants of osmotic minipumps, containing 0.55 mg/ml (0.05 $\mu g \cdot g^{-1} \cdot d^{-1}$) or 0.11 mg/ml (0.01 $\mu g \cdot g^{-1} \cdot d^{-1}$) rbIGF-I or saline, respectively. Temperature was maintained at 14 ± 1° throughout the experiment. Fish were held in the Life Sciences Building courtyard and fed *ad libitum* two to three times daily. Each week the fish were anesthetized and length and weight were measured.

Experiment 4. One-year-old juvenile coho salmon with initial weights of 28–40 g were randomly separated into three groups. On March 28, each group received implants of osmotic minipumps, containing 1.0 mg/ml (0.12 $\mu g \cdot g^{-1} \cdot d^{-1}$) or 0.5 mg/ml (0.06 $\mu g \cdot g^{-1} \cdot d^{-1}$) rbIGF-I or saline vehicle, respectively. Temperature was maintained at 15 ± 1° throughout the experiment. Fish were held in the Life Sciences Annex Building where lighting was provided by overhead fluorescent bulbs. The photoperiod was a constant 24 hr of daylight throughout the experiment (the result of a failure in the light-timing controls). Each week the fish were anesthetized and length and weight were measured. Fish were fed 0.6% body wt per day adjusted weekly.

Experiment 5. One-year-old juvenile coho salmon with initial weights of 31–42 g were randomly separated into three groups. On June 7, each group received implants of osmotic minipumps, containing 0.88 mg/ml (0.08 $\mu g \cdot g^{-1} \cdot d^{-1}$) or 0.22 mg/ml (0.02 $\mu g \cdot g^{-1} \cdot d^{-1}$) rbIGF-I or saline vehicle, respectively. Temperature was maintained at 15 ± 1° throughout the experiment. Fish were held in the Life Sciences Annex Building; the photoperiod was a simulated natural photoperiod throughout the experiment. The tank was divided into three equal compartments with plastic mesh to separate the groups. Each group was fed 0.5% body wt per day, adjusted weekly. Each week the fish were anesthetized and length and weight were measured.

Length was measured from the tip of the snout to

the fork of the caudal fin (fork length). Fish were dried with a chamois cloth prior to weighing. Blood was collected from the caudal vessels using a syringe treated with ammonium heparin. Plasma was obtained by centrifugation at 5000 *g* for 1 min and stored at −80°. In experiment 5, liver and intestine weights, intestine length, and muscle moisture content were measured. The intestine (last pyloric cecum to anus) was removed, split longitudinally, and rinsed with saline. The organ was then blotted dry and its weight and length were measured. Muscle moisture content was measured by drying a 2.5- to 4-g piece of upper-lateral, white muscle to a constant weight at 60° (Busacker *et al.*, 1990).

Analytical methods. Plasma glucose was assayed in 10-μl samples by measuring the production of NADH in the presence of hexokinase and glucose-6-phosphate dehydrogenase at 350 nm in a recording spectrophotometer (Perkin–Elmer, Wilton, CT). Growth rate of individual fish was calculated as $((\ln W_{t_2} - \ln W_{t_1})/(t_2 - t_1)) \cdot 100$, where W = weight of an individual at the beginning (t_1) or the end of the experiment (t_2). Only fish that survived the entire duration of the study were used to calculate growth rates. Calculation of actual doses of IGF-I are based on the initial weights of the animals. Statistical comparisons were by the Student *t* test (two groups) or one-way ANOVA (three groups) followed by Dunnett's test, using the CRISP statistical program (CRUNCH, Berkeley, CA). Variances were found to be homogeneous in all groups in which statistical comparisons were made (F_{max} test). Differences were considered statistically significant when $P < 0.05$ unless stated otherwise.

RESULTS

Experiment 1. Mortality occurred in the rbIGF-I-treated group (average dose, 0.41 [range, 0.32–0.51] $\mu g \cdot g^{-1} \cdot d^{-1}$) within 48 hr of implantation. After 6 days of treatment, four fish had died and four of the remaining fish were swimming abnormally. There was substantial hypoglycemia in the surviving rbIGF-I-treated fish relative to the controls (Table 1). None of the control fish died during the 6 days of treatment or in any subsequent experiment.

Experiment 2. Two-year-old coho salmon receiving a dose of 0.16 (0.13–0.19) $\mu g \cdot g^{-1} \cdot d^{-1}$ rbIGF-I or greater (fish smaller than 200 g) died within 1 week of receiving the minipump implant. The remaining rbIGF-I-treated fish (0.09 [0.07–0.12] $\mu g \cdot g^{-1} \cdot d^{-1}$) grew significantly faster than the control fish (Table 2) over the 25 days of treatment. Linear growth rate doubled and growth rate in weight increased by 40% in the rbIGF-I-treated group relative to the control group. There was no significant difference in plasma glucose between the two groups.

TABLE 1
EFFECT OF BOVINE rbIGF-I (0.4 $\mu g \cdot g^{-1} \cdot d^{-1}$ FOR 6 DAYS) ON MORTALITY AND PLASMA GLUCOSE OF 1-YEAR-OLD COHO SALMON IN FRESH WATER (EXPERIMENT 1)

	Mortality	Plasma glucose (m*M*)
Control (n = 10)	0/10	4.3 ± 0.1
rbIGF-I (n = 5)	5/10	2.1* ± 0.7

* Significantly different from control group ($P < 0.05$, Student's *t* test).

Experiment 3. One-year-old coho salmon were fed *ad libitum* twice daily for the 21 days of the study. There was no mortality in rbIGF-I-treated (0.01 [0.008–0.13] and 0.05 [0.04–0.06] $\mu g \cdot g^{-1} \cdot d^{-1}$) or control fish, and there was no significant effect of rbIGF-I on growth rate or plasma glucose (Table 3). Growth rate of the control group in this experiment was twice that of the controls in experiment 2.

Experiment 4. One-year-old coho salmon were fed a limited ration (0.6% body wt per day) for the 21 days of the study. Although low, the growth rate was positive in the control group (Table 4, Fig. 1). At 0.06 [0.05–0.07] and 0.12 [0.10–0.14] $\mu g \cdot g^{-1} \cdot d^{-1}$, rbIGF-I substantially and significantly increased growth rate relative to the controls (Table 4, Fig. 1). Growth rates in length and weight were three- and fourfold higher, respectively, in rbIGF-I-treated fish than in controls (Table 4). Plasma glucose in both rbIGF-I-treated groups was significantly lower than in controls.

Experiment 5. One-year-old coho salmon were fed a limited ration (0.5% body wt per day) for the 21 days of the study, and each group was housed separately to ensure

TABLE 2
EFFECT OF BOVINE rbIGF-I TREATMENT FOR 25 DAYS ON MORTALITY, GROWTH RATE, AND PLASMA GLUCOSE OF 2-YEAR-OLD COHO SALMON IN FRESH WATER[a]

	Mortality	Growth rate		Plasma glucose (m*M*)
		Length (% · d^{-1})	Weight (% · d^{-1})	
Control (n = 8)	0/8	0.16 ± 0.01	0.97 ± 0.04	4.24 ± 0.25
rbIGF-I (n = 4)	4/8	0.30* ± 0.03	1.35* ± 0.02	3.30 ± 0.43

[a] Fish smaller than 200 g in the rbIGF-I (effective dose >0.13 μg · g^{-1} · d^{-1}) treated group died; dose in remaining fish was 0.09 μg · g^{-1} · d^{-1} (experiment 2).

* Significantly different from control group ($P < 0.05$, Student's t test).

equal food consumption. Linear growth rate was more than threefold higher in the 0.08 (0.07–0.10) μg · g^{-1} · d^{-1} rbIGF-I group than in the controls (Table 5). Although growth rate in weight was doubled relative to controls, this change was not statistically significant. At 0.02 (0.01–0.03) μg · g^{-1} · d^{-1}, rbIGF-I had a slight but statistically insignificant effect on growth (Table 5). A summary of the relative effects of rbIGF-I on linear growth rate in ration-limited fish indicates a dose-dependent effect (experiments 4 and 5, Fig. 2). In experiment 5, there was no significant effect of rbIGF-I treatment on intestinal length or muscle moisture content ($P \geq 0.4$, Table 6). In comparison to the control group, relative liver weight was 17% lower in the high dose rbIGF-I group, but 7% higher in the low dose group ($P = 0.06$, one-way ANOVA). There was a trend for increasing relative intestinal weight with increasing rbIGF-I (23% increase at the highest dose), but this difference was not statistically significant ($P = 0.15$).

DISCUSSION

Although there is only a handful of published studies on the effect of IGF-I on growth of vertebrates, it appears that the effects are most readily observed in animals with impaired endogenous production of IGF-I (and associated reductions in growth rate). IGF-I stimulates growth in hypophysectomized and diabetic rats (Schoenle *et al.*, 1982; Skottner *et al.*, 1987; Scheiwiller *et al.*, 1986; Guler *et al.*, 1988, 1989), which have low circulating levels of IGF-I and inhibited growth. However, Hizuka *et al.* (1986) observed a slight (22%) increase in growth rate in 23-day-old intact rats as a result of IGF-I infusion. Although miniature poodles have lower levels of IGF-I than king poodles (Eigenmann *et al.*, 1984), IGF-I apparently does not stimulate growth of miniature poodles (Guler *et al.*, 1989). These somewhat conflicting results do not clearly define the limitations of exogenous IGF-I treatment on growth of vertebrates, which may be dependent on the species be-

TABLE 3
EFFECT OF rbIGF-I ON MORTALITY, GROWTH RATE, AND PLASMA GLUCOSE IN 1-YEAR-OLD COHO SALMON (EXPERIMENT 3)

	Mortality	Growth rate		Plasma glucose (m*M*)
		Length (% · d^{-1})	Weight (% · d^{-1})	
Control	0/10	0.26 ± 0.02	0.91 ± 0.06	4.53 ± 0.27
rbIGF-I (0.01 μg · g^{-1} · d^{-1})	0/10	0.21 ± 0.03	0.79 ± 0.08	4.58 ± 0.28
rbIGF-I (0.01 μg · g^{-1} · d^{-1})	0/10	0.25 ± 0.02	0.90 ± 0.05	4.30 ± 0.27

TABLE 4
EFFECT OF rbIGF-I ON MORTALITY, GROWTH RATE, AND PLASMA GLUCOSE IN 1-YEAR-OLD COHO SALMON MAINTAINED ON LIMITED RATION[a]

	Mortality	Growth rate		Plasma glucose (m*M*)
		Length (% · d^{-1})	Weight (% · d^{-1})	
Control	0/10	0.06 ± 0.03	0.21 ± 0.14	4.67 ± 0.24
rbIGF-I (0.06 μg · g^{-1} · d^{-1})	0/10	0.20* ± 0.02	0.86* ± 0.10	3.61* ± 0.18
rbIGF-I (0.12 μg · g^{-1} · d^{-1})	4/10	0.21* ± 0.02	0.74* ± 0.12	2.97* ± 0.13

[a] 0.6% body wt per day; experiment 4.
* Significantly different from control group ($P < 0.05$, one-way ANOVA followed by Dunnett's test).

ing examined, the developmental stage, and the environmental conditions. For example, production of IGF-binding proteins (IGF-BPs), which affect the availability of IGF-I to target tissues, are affected by diet and various other factors (Rosenfeld, 1990).

In the present study, rbIGF-I caused significant stimulation of growth rate, but only in animals with submaximal growth rates (experiments 2, 4, and 5; length-specific growth rate of control fish, 0.04–0.16 % · d^{-1}). There was no effect of IGF-I on rapidly growing coho salmon (experiment 3; length-specific growth rate of control fish, 0.26 % · d^{-1}). In experiments 4 and 5, growth rate was reduced by feeding a reduced ration to the fish. Fasting and protein restriction of rats lead to reduced hepatic GH receptor function and binding (Baxter *et al.*, 1982; Maes *et al.*, 1983, 1984; Bornfeldt *et al.*, 1989), diminished IGF-I production, and decreased growth rate (Phillips and Young, 1976; Maes *et al.*, 1983, 1984; Moats-Staats *et al.*, 1989). It seems likely that IGF-I infusion supplemented the reduced endogenous IGF-I production in ration-limited fish. The lower growth of control fish in experiment 2 (fed *ad libitum* once daily) may have been due to several factors, including age, the single daily feeding, and/or the larger initial body size, but the stimulation by rbIGF-I suggests an action similar to that seen in experiments 4 and 5. Conversely, failure to observe effects of rbIGF-I in rapidly growing coho salmon may have arisen from the high endogenous production of GH and IGF-I.

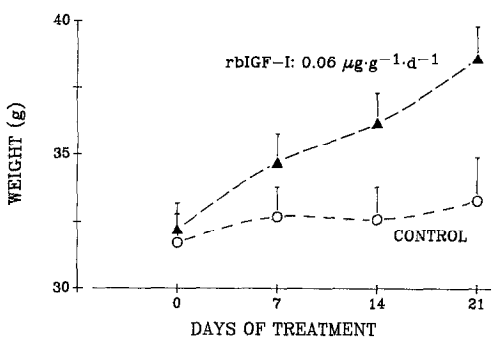

FIG. 1. Time course of changes in body weight of coho salmon in response to intraperitoneal infusion of rbIGF-I. Control group received intraperitoneal infusion of vehicle (saline). Both groups were maintained on limited rations (0.6% body wt per day). The values from the 0.12 μg · g^{-1} · d^{-1} group substantially overlap those from the 0.06 μg · g^{-1} · d^{-1} group and, for clarity, are not presented. Values are means ± SE; sample size was 10 fish at each time point for each group.

Observations from experiments 2 and 4 indicated that IGF-I-treated fish were more aggressive in competing for food than untreated fish and that this may have contributed to some of the observed differences in growth rate in these experiments. The results of experiment 5, in which groups were separated, indicate that the effect of IGF-I on linear growth rate is not a function of increased food intake. The relative ability of IGF-I to stimulate growth in weight, however, was decreased in experiment 5 (three- to fourfold higher in experiment 4 [$P < 0.05$], twofold higher in experiment 5 but not statistically significant), indicating that

TABLE 5
EFFECT OF rbIGF-I ON MORTALITY, GROWTH RATE, AND PLASMA GLUCOSE IN 1-YEAR-OLD COHO SALMON MAINTAINED ON LIMITED RATION[a]

	Mortality	Growth rate		Plasma glucose (m*M*)
		Length (% · d^{-1})	Weight (% · d^{-1})	
Control	0/10	0.04 ± 0.03	0.16 ± 0.14	3.72 ± 0.13
rbIGF-I (0.02 μg · g^{-1} · d^{-1})	0/10	0.06 ± 0.02	0.20 ± 0.10	3.58 ± 0.43
rbIGF-I (0.08 μg · g^{-1} · d^{-1})	0/10	0.14* ± 0.02	0.36 ± 0.12	2.35* ± 0.35

[a] 0.5% body wt per day; experiment 5.
* Significantly different from control group ($P < 0.05$, one-way ANOVA followed by Dunnett's test).

at least some of the increase in weight observed in experiments 2 and 4 may have been due to increased food consumption. Guler *et al.* (1989) observed increased food consumption of hypophysectomized rats infused with IGF-I for 18 days. Increases in weight were not due to increased water content, as muscle moisture content did not differ as a result of rbIGF-I treatment (Table 6).

Saunders *et al.* (1985) have demonstrated that continuous light administered during a declining photoperiod can result in increased growth of Atlantic salmon (*Salmo salar*). It seems unlikely that the use of continuous light in experiment 4, however, had a significant impact on growth of control or treated fish since the growth rates of fish in experiments 4 and 5 (the latter under simulated natural photoperiod) were similar.

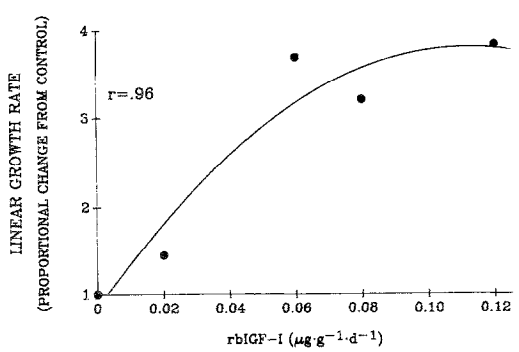

FIG. 2. Effect of rbIGF-I on specific growth rate in length of coho salmon maintained on limited ration (0.5–0.6% body wt per day). Results are expressed as the proportional change from the respective control group in experiments 4 and 5 (see text for details).

Failure to observe an effect of continuous light may have been due to the low ration level employed, or to the application of continuous light at a time when natural photoperiod was increasing (spring).

A number of studies indicate that GH can increase growth rate in intact fish, including rapidly growing juvenile coho salmon (see Donaldson *et al.*, 1979, for review; Down *et al.*, 1988, 1989; Moriyama and Kawauchi, 1990). Using osmotic minipumps as in the present study, Down *et al.* (1988) found that 0.1 μg · g^{-1} · d^{-1} recombinant bovine growth hormone increased weight- and length-specific growth rate of coho salmon by 2.5-fold. Although it has not been widely investigated, the ability of exogenous growth hormone to stimulate growth may also be less apparent when growth conditions are optimal (see, for instance, the possible effect of temperature on the efficacy of growth hormone treatment in salmonids, [Danzmann *et al.*, 1990]). Nonetheless, these comparisons indicate that exogenous growth hormone may be more effective than recombinant bovine IGF-I in increasing growth in fish. Although further studies are required, these comparisons indicate that IGF-I may carry out only some of the growth-stimulating functions of growth hormone in salmon.

In the present study, high doses of rbIGF-I (>0.13 μg · g^{-1} · d^{-1}) caused hypoglycemia and death (experiments 1 and 2). In mammals, experimentally increased serum levels of free IGF-I (i.e., not bound

TABLE 6
EFFECT OF rbIGF-I ON LIVER WEIGHT, INTESTINAL WEIGHT, INTESTINAL LENGTH, AND MUSCLE MOISTURE CONTENT IN 1-YEAR-OLD COHO SALMON MAINTAINED ON LIMITED RATION[a]

	Liver weight (% body wt)	Intestine weight (% body wt)	Intestine length (% body length)	Muscle moisture (%)
Control	0.89 ± 0.04	0.29 ± 0.02	41.6 ± 1.0	73.6 ± 0.6
rbIGF-I (0.02 $\mu g \cdot g^{-1} \cdot d^{-1}$)	0.95 ± 0.03	0.30 ± 0.02	41.1 ± 1.3	73.6 ± 0.4
rbIGF-I (0.08 $\mu g \cdot g^{-1} \cdot d^{-1}$)	0.73 ± 0.07	0.35 ± 0.03	40.9 ± 1.2	72.2 ± 1.3

[a] 0.5% body wt per day; experiment 5.

to IGF-BPs) can induce severe hypoglycemia (Guler *et al.*, 1988), presumably by interacting with insulin receptors (Froesch and Zapf, 1985). Recently, several IGF-BPs have been identified in coho salmon plasma (K. M. Kelley and K. Siharath, unpublished results), and thus it is possible that the higher doses of rbIGF-I similarly resulted in IGF-I levels in excess of circulating IGF-BPs. Increased growth rate of coho salmon induced by rbIGF-I administration (experiments 2, 4, and 5) was associated with slight hypoglycemia. This is not a universal finding, however, as Guler *et al.* (1989) report no effect of IGF-I on plasma glucose in hypophysectomized rats despite a dramatic effect on growth rate.

IGF-I treatment can also have differential effects on the growth of various organs in mammals (Guler *et al.*, 1988, 1989). Collie and Stevens (1985) found that growth hormone treatment increased the area-specific intestinal weight of coho salmon. However, the present study indicates that relative liver and intestinal weights of coho salmon are not affected by rbIGF-I (Table 6).

Skyrud *et al.* (1989) found that multiple injections of mammalian IGF-I in brook trout resulted in hypoglycemia and poor growth. These authors suggested that there was a structural difference between mammalian and brook trout IGF-I that precluded binding of mammalian IGF-I to the brook trout receptor. This is not supported by recent *in vitro* studies in which recombinant bovine IGF-I stimulated sulfate incorporation into cartilage from several teleosts (Duan and Hirano, 1990; McCormick *et al.*, 1991; Gray and Kelley, 1991; see review by Bern *et al.*, 1991) or by the positive effects of constant infusion of rbIGF-I on growth of coho salmon in the present study. Furthermore, Cao *et al.* (1990) reported that the deduced amino acid sequence of coho salmon IGF-I has an 80% sequence similarity with mammalian IGF-I. It is possible that the negative effect of IGF-I on growth of brook trout was the result of the route of administration used by Skyrud *et al.* (1989). Bolus injection of IGF-I induces hypoglycemia and poor growth in mammals, whereas constant infusion at similar average daily doses promotes growth (Froesch and Zapf, 1985; Scheiwiller *et al.*, 1986; Guler *et al.*, 1988, 1989).

Studies using heterologous radioimmunoassays or radioreceptor assays in several teleost species yield an inconsistent picture of the presence and regulation of circulating "IGF-I-like" factors (van den Brande *et al.*, 1974; Furlanetto *et al.*, 1977; Shapiro and Pimstone, 1977; Wilson and Hinz, 1982; Daughaday *et al.*, 1985; Lindahl *et al.*, 1985; Drakenberg *et al.*, 1989). However, Funkenstein *et al.* (1990) reported that GH treatment increased (human) IGF-I immunoreactivity in the plasma of sea bream (*Sparus aurata*). In addition, Cao *et al.* (1990) found that coho salmon liver IGF-I mRNA is responsive to *in vivo* growth hormone treatment. The present results indicate that mammalian IGF-I can increase growth rate of coho salmon under conditions of limited ration. In combination with data indicating that mammalian IGF-I

increases the *in vitro* sulfate incorporation in cartilage of coho salmon (McCormick *et al.*, 1991) and other teleosts (Duan and Hirano, 1990; Gray and Kelley, 1991), the present data support a role for endogenous IGF-I as an important mediator of some of the actions of GH in promoting and regulating growth in teleosts.

ACKNOWLEDGMENTS

We thank the Monsanto Corp. (St. Louis, MO) for generously supplying us with rbIGF-I. This work is a result of research sponsored in part by NOAA, National Sea Grant College Program, Department of Commerce, under Grant NA85AA-D-SG-140, through the California Sea Grant College Program, in part by the California State Resources Agency, Project R/F-117, and in part by NSF Grant DCB 84-0549. We thank the California Department of Fish and Game for supplying fish used in this study.

REFERENCES

Baxter, R. C., Bryson, J. M., and Turtle, J. R. (1982). The effect of fasting on liver receptors for prolactin and growth hormone. *Metabolism* **30**, 1086–1090.

Bern, H. A., McCormick, S. D., Kelley, K. M., Gray, E. S., Nishioka, R. S., Madsen, S. S., and Tsai, P. I. (1991). Insulin-like growth factors "under water": Role in growth and function of fish and other poikilothermic vertebrates. *In* "Insulin-like Growth Factors/Somatomedins" (E. M. Spencer, Ed.), pp. 85–96. Elsevier Press, New York.

Bornfeldt, K. E., Arnqvist, H. J., Enberg, B., Mathews, L. S., and Norstedt, G. (1989). Regulation of insulin-like growth factor I and growth hormone receptor gene expression by diabetes and nutritional state in rat tissues. *J. Endocrinol.* **122**, 651–656.

Busacker, G. P., Adelman, I. R., and Goolish, E. M. (1990). *In* "Methods for Fish Biology," (C. B. Schreck and P. B. Moyle, Eds.), pp. 363–388. American Fisheries Society, Bethesda.

Cao, Q.-P., Duguay, S., Plisetskaya, E., Steiner, D. F., and Chan, S. J. (1990). Nucleotide sequence and growth hormone-regulated expression of salmon insulin-like growth factor I RNA. *Mol. Endocrinol.* **3**, 2005–2010.

Collie, N. L., and Stevens, J. J. (1985). Hormonal effects on L-proline transport in coho salmon *Oncorhynchus kisutch* intestine. *Gen. Comp. Endocrinol.* **59**, 399–409.

Danzmann, R. G., Van der Kraak, G. J., Chen, T. T., and Powers, D. A. (1990). Metabolic effects of bovine growth hormone and genetically engineered rainbow trout growth hormone in rainbow trout (*Oncorhynchus mykiss*) reared at high temperature. *Can. J. Fish. Aquat. Sci.* **47**, 1292–1301.

Daughaday, W. H., Kapadia, M., Yanow, C. E., Fabrick, K., and Mariz, I. K. (1985). Insulin-like growth factors I and II of nonmammalian sera. *Gen. Comp. Endocrinol.* **59**, 316–325.

Daughaday, W. H., and Rotwein, P. (1989). Insulin-like growth factor I and II. Peptide, messenger ribonucleic acid and gene structures, serum, and tissue concentrations. *Endocr. Rev.* **10**, 68–91.

Donaldson, E. M., Fagerlund, U. H. M., Higgs, D. A., and McBride, J. R. (1979). Hormonal enhancement of growth. *In* "Fish Physiology" (W. S. Hoar, D. J. Randall, and J. R. Brett, Eds.), Vol. 8, pp. 456–597. Academic Press, New York.

Down, N. E., Donaldson, E. M., Dye, H. M., Langley, K. E., and Souza, L. M. (1988). Recombinant bovine somatotropin more than doubles the growth rate of coho salmon (*Oncorhynchus kisutch*) acclimated to seawater and ambient winter conditions. *Aquaculture* **68**, 141–155.

Down, N. E., Donaldson, E. M., Dye, H. M., Boone, T. C., Langley, K. E., and Souza, L. M. (1989). A potent analog of recombinant bovine somatotropin accelerates growth in juvenile coho salmon (*Oncorhynchus kisutch*). *Can. J. Fish. Aquat. Sci.* **46**, 178–183.

Drakenberg, K., Sara, V. R., Lindahl, K. I., and Kewish, B. (1989). The study of insulin-like growth factors in tilapia, *Oreochromis mossambicus*. *Gen. Comp. Endocrinol.* **74**, 173–180.

Duan, C., and Hirano, T. (1990). Stimulation of ^{35}S-sulfate uptake by mammalian insulin-like growth factors I and II in cultured cartilages of the Japanese eel, *Anguilla japonica*. *J. Exp. Zool.* **256**, 347–350.

Eigenmann, J. E., Patterson, D. F., and Froesch, E. R. (1984). Body size parallels insulin-like growth factor I levels but not growth hormone secretory capacity. *Acta Endocrinol.* **106**, 448–453.

Froesch, E. R., and Zapf, J. (1985). Insulin-like growth factors and insulin: Comparative aspects. *Diabetologia* **28**, 485–493.

Funkenstein, B., Silbergeld, A., Cavari, B., and Laron, Z. (1990). Growth hormone increases plasma levels of insulin-like growth factor (IGF-I) in a teleost, the gilthead bream (*Sparus auratus*). *J. Endocrinol.* **120**, R19–R21.

Furlanetto, R. W., Underwood, J. J., Van Wyk, J. J., and D'Ercole, A. J. (1977). Estimation of somatomedin-C levels in normals and patients with pituitary disease by radioimmunoassay. *J. Clin. Invest.* **60**, 648–657.

Gray, E. S., and Kelley, K. M. (1991). Growth regulation in the gobiid teleost, *Gillichthys mirabilis*:

Roles of growth hormone, hepatic growth hormone receptors and insulin-like growth factor I. *J. Endocrinol.* **130,** in press.

Guler, H. P., Zapf, J., Scheiwiller, E., and Froesch, E. R. (1988). Recombinant human insulin-like growth factor I stimulates growth and has distinct effects on organ size in hypophysectomized rats. *Proc. Natl. Acad. Sci. USA* **85,** 4889–4893.

Guler, H. P., Zapf, J., Binz, K., and Froesch, E. R. (1989). Growth promotion using recombinant insulin-like growth factor-1. *In* "Biotechnology in Growth Regulation" (R. B. Heap, C. G. Prosser, and G. E. Lamming, Eds.), pp. 119–122. Butterworths, Stoneham.

Hizuka, N., Takano, K., Shizume, K., Asakawa, K., Miyakawa, M., Tanaka, I., and Horikawa, R. (1986). Insulin-like growth factor I stimulates growth in normal growing rats. *Eur. J. Pharmacol.* **125,** 143–146.

Isaksson, O. G., Lindahl, A., Nilsson, A., and Isgaard, J. (1987). Mechanism of the stimulatory effect of growth hormone on longitudinal bone growth. *Endocr. Rev.* **8,** 426–438.

Lindahl, K. I., Sara, V., Fridberg, G., and Nishimiya, T. (1985). The presence of somatomedin in the Baltic salmon, *Salmo salar,* with special reference to smoltification. *Aquaculture* **45,** 117–183.

Maes, M., Underwood, L. E., and Ketelslegers, J.-M. (1983). Plasma somatomedin-C in fasted and refed rats: Close relationship with changes in liver somatogenic but not lactogenic binding sites. *J. Endocrinol.* **97,** 243–252.

Maes, M., Underwood, L. E., and Ketelslegers, J.-M. (1984). Low serum somatomedin-C in protein deficiency: Relationship with changes in liver somatogenic lactogenic binding sites. *Mol. Cell. Endocrinol.* **37,** 301–309.

McCormick, S. D., Tsai, P. I., Kelley, K. M., Nishioka, R. S., and Bern, H. A. (1991). Hormonal control of sulfate incorporation in branchial cartilage of coho salmon: role of IGF-I. *J. Exp. Zool.,* **261,** in press.

Moats-Staats, B. M., Brady, J. L., Underwood, L. E., and D'Ercole, J. (1989). Dietary protein restriction in artificially reared neonatal rats causes a reduction of insulin-like growth factor I gene expression. *Endocrinology* **125,** 2368–2374.

Moriyama, S., and Kawauchi, H. (1990). Growth stimulation of juvenile salmonids by immersion in recombinant salmon growth hormone. *Bull. Jap. Soc. Scient. Fish.* **56,** 31–34.

Phillips, L. S., and Young, H. S. (1976). Nutrition and somatomedin. I. Effects of fasting and refeeding on serum somatomedin activity and cartilage growth activity in rats. *Endocrinology* **99,** 304–314.

Rosenfeld, R. G., Lawson, G., Pham, H., Youngman, O. H., Conover, C., DeLeon, D. D., Donovan, S. M., Ocrant, I., and Giudice, L. (1990). Insulin-like growth factor—Binding proteins. *Rec. Prog. Horm. Res.* **46,** 99–159.

Scheiwiller, E., Guler, H. P., Merryweather, J., Scandella, C., Maerki, W., Zapf, J., and Froesch, E. R. (1986). Growth restoration of insulin-deficient diabetic rats by recombinant human insulin-like growth factor I. *Nature* **323,** 169–171.

Schoenle, E., Zapf, J., Humbel, R., and Froesch, E. R. (1982). Insulin-like growth factor I stimulates growth in hypophysectomized rats. *Nature* **296,** 252–253.

Shapiro, B., and Pimstone, B. L. (1977). A phylogenetic study of sulphation factor activity in 26 species. *J. Endocrinol.* **74,** 129–135.

Skottner, A., Clark, R. G., Robinson, I. C. A. F., and Fryklund, L. (1987). Recombinant human insulin-like growth factor: Testing the somatomedin hypothesis in hypophysectomized rats. *J. Endocrinol.* **112,** 123–132.

Skyrud, T., Andersen, O., Alestrom, P., and Gautvik, K. M. (1989). Effects of recombinant human growth hormone and insulin-like growth factor I on body growth and blood metabolites in brook trout (*Salvelinus fontinalis*). *Gen. Comp. Endocrinol.* **75,** 247–255.

van Bull-Offers, S., Ueda, I., and van den Brande, J. L. (1986). Biosynthetic somatomedin C (SM-C/IGF-I) increases the length and weight of Snell dwarf mice. *Pediatr. Res.* **20,** 825–827.

van den Brande, J. L., Kootte, F., Tielenburg, R., van der Wilk, M., and Huyser, T. (1974). Studies on plasma somatomedin activity in different animal species. *Acta Endocrinol.* **75,** 243–248.

Weatherley, A. H., and Gill, H. S. (1990). "The Biology of Fish Growth," pp 1–443. Academic Press, San Diego.

Wilson, D. W., and Hinz, R. L. (1982). Inter-species comparison of somatomedin structure using immunological probes. *J. Endocrinol.* **95,** 50–64.