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Hormonal Control of Sulfate Uptake by Branchial Cartilage of Coho Salmon: Role of IGF-I

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ABSTRACT The direct hormonal control of sulfate uptake by cartilage matrix of coho salmon was examined by exposing branchial cartilage to 1 $\mu Ci \cdot ml^{-1}$ $^{35}SO_4$ for 48 hours at 15°C in a defined medium. Sulfate uptake occurred primarily in cartilage (rather than bone) and the amount of specific uptake was similar in epibranchial and ceratobranchial cartilages. Intact and hypophysectomized coho salmon starved for 22 days had equivalent in vitro sulfate uptake, which in both cases were 30% of the uptake seen in branchial cartilage of fed, intact controls. In branchial cartilage from starved coho salmon, in vitro exposure to recombinant bovine insulin-like growth factor I (rbIGF-I) at 1, 10, 100, and 1,000 $ng \cdot ml^{-1}$ caused a dose-dependent increase in sulfate uptake, with a maximum 3-fold increase over control at 1,000 $ng \cdot ml^{-1}$ rbIGF-I. Coho salmon insulin (1, 10, 100, and 1,000 $ng \cdot ml^{-1}$) resulted in a maximum 30% increase in sulfate uptake at the highest dose. Growth hormone and triiodo-L-thyronine had no direct effect on in vitro sulfate uptake. The results indicate that IGF-I has direct effects on coho salmon cartilage and may be an important regulator of growth in salmon and other teleosts.

From the observations that growth hormone (GH) promotes sulfate uptake in cartilage in vivo but not in vitro, and that GH injection increases the capacity of plasma to increase sulfate uptake in vitro, a mediator of the growth-promoting action of growth hormone was hypothesized (Salmon and Daughaday, '57). Subsequent research has demonstrated that the most important of these somatomedins is insulin-like growth factor I (IGF-I), an endocrine and paracrine factor that is produced in the liver and a variety of other tissues (Daughaday and Rotwein, '89). Although the relative roles of circulating IGF-I, growth hormone, and other growth factors in controlling mammalian growth rate is controversial (see Isaksson et al. '87; Daughaday and Rotwein, '89), it is clear that IGF-I is involved in the regulation of bone growth and whole-animal growth rates.

The role of IGF-I in the growth of teleost fishes is less clear. Studies using heterologous radioimmunoassays or radioreceptor assays in several teleost species do not yield a consistent picture of the presence or regulation of circulating "IGF-I-like" factors (Furlanetto et al., '77; Wilson and Hinz, '82; Daughaday et al., '85; Lindahl et al., '85; Drakenberg et al., '89; Funkenstein et al., '90). However, the IGF-I gene from coho salmon (*Oncorhynchus kisutch*) liver has recently been cloned (Cao et al., '90); the deduced amino acid composition has 80% sequence similarity to mammalian IGF-I, and liver IGF-I mRNA is responsive to in vivo growth hormone treatment.

Recent studies have also suggested that somatomedins may be important in the regulation of fish growth. Komourdjian and Idler ('78) reported that a combination of GH and thyroxine in vitro increased sulfate uptake in rainbow trout (*Oncorhynchus mykiss*) bone when co-incubated with liver slices. Duan and Inui ('90b) found that a GH-induced plasma factor increases sulfate uptake by isolated eel cartilage. Furthermore, mammalian IGF-I stimulates in vitro sulfate uptake in eel cartilage (Duan and Hirano, '90). However, Skyrud et al. ('89) found that multiple injections of mammalian IGF-I in brook trout (*Salvelinus fontinalis*) resulted in slower growth than controls. In this study we investigate the endocrine factors regulating in vitro sulfate uptake in a salmonid teleost.

MATERIALS AND METHODS

Animals

Coho salmon *(Oncorhynchus kisutch)* obtained from Iron Gate Hatchery (California Department of Fish and Game) were reared under standard con-

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ditions as previously described (natural photoperiod, 10–14°C; McCormick and Bern, '89). Fish were fed Oregon Moist pellet (Moore-Clarke, LaConner, WA) ad libitum twice daily. Post-smolt juveniles were hypophysectomized by the orbital method (Nishioka, '80) and maintained in fresh water.

Solutions and hormones

Preincubation medium was Minimal Essential Medium (MEM; Gibco) with Hanks' salts, 25 mM HEPES buffer, 200 U · ml^{-1} penicillin and 200 μg · ml^{-1} streptomycin (pH 7.6). Incubation medium was MEM with Earle's salts, 1 μCi · ml^{-1} [^{35}S]sulfate (Amersham, Arlington Heights, IL), 50 U · ml^{-1} penicillin, and 50 μg · ml^{-1} streptomycin, adjusted to pH 7.6 after gassing. Stock solutions of hormones were prepared in the following concentrations with sterile solvents: recombinant bovine insulin-like growth factor I (gift from Monsanto, St. Louis, MO; rbIGF-I content was greater than 95% as determined by high pressure liquid chromatography, and contained no detectable IGF-II), 0.5 mg · ml^{-1} in MEM; native coho salmon insulin (gift from Dr. E. Plisetskaya, University of Washington), 0.5 mg · ml^{-1} in MEM, native chum salmon growth hormone (gift from Prof. H. Kawauchi, Kitasato University) 0.25 mg · ml^{-1} in 0.01 N NaOH, 0.8% NaCl; triiodo-L-thyronine (T_3, sodium salt, Sigma T 2752), 10 mg · ml^{-1} in 0.01 N NaOH, 0.08% NaCl. Basic stock solutions were neutralized with HCl prior to use. All solutions, including controls, contained the same amount of hormone solvent as was used for the maximum dose.

Sulfate uptake

Branchial cartilage was isolated by the method of Duan and Inui ('90a), and sulfate uptake was measured by a modification of the method of Ishii and Kikuyama ('84). Fish were killed by a blow to the head, and the first three pairs of gill arches were removed and placed in preincubation medium on ice. The skin and gill tissue were removed and the epibranchial and ceratobranchial were separated. The cartilage could be easily distinguished from bone by its translucent appearance. A cut was made just distal to the cartilage/bone interface, and the cartilage was placed in preincubation medium. One ceratobranchial cartilage from each individual was placed in preincubation medium in boiling water for 10 minutes. The remaining cartilages were kept in preincubation medium for 0.5–1 hours at 15°C. Cartilage pieces were then placed in incubation media (with or without hormone) with 1 μCi · ml^{-1} [^{35}S]sulfate. Tissue was incubated for up to 48 hours at 15°C under 95% O_2, 5% CO_2. Following incubation the cartilage was removed, blotted dry, and placed in saturated Na_2SO_4 solution for at least 2 hours. Tissue was then placed in running tap water for at least 1 hour, and then rinsed 4 times with distilled water. The tissue was dried to a constant weight at 60°C in a convection oven (for at least 3 hours). Dried cartilage was weighed to the nearest μg and placed in a 7-ml scintillation vial with 0.5 ml 99% formic acid heated to 60°C overnight. Scintillation vials were vortexed, 3.5 ml scintillation fluid was added, vials were vortexed again, and disintegrations per minute (dpm) were counted in a Beckman 5000 liquid scintillation counter. Nonspecific uptake (boiled cartilages) was subtracted from total uptake; the resulting values for specific uptake were expressed as dpm per μg dry weight of tissue. Preliminary results indicated a parabolic increase in sulfate uptake through the first 48 hours, similar to that reported by Duan and Inui ('90b). An endpoint of 48 hours was chosen for routine analysis of sulfate uptake.

Autoradiography

To visualize regions of ^{35}S-sulfate uptake by the branchial arch, whole branchial arches were dissected from their soft tissues, leaving intact the connective tissue joint between the epibranchial and ceratobranchial bones, and incubated as above for 48 hours. After incubation, the arches were placed in vials containing saturated Na_2SO_4 solution and constantly agitated for 4 hours. The arches were then blotted dry, placed on cardboard, covered with plastic wrap, and placed against XAR autoradiography film (Eastman Kodak, Rochester, NY) in a tightly clamped X-ray exposure folder. After exposure for 4 d at −80°C, the film was developed on a Konica auto processor (model QK-130A).

Statistical analysis

All statistical comparisons were by the nonparametric Wilcoxon test followed by the Mann-Whitney test using the CRISP statistical program (CRUNCH, Berkeley CA). Statistical significance was $P < .05$ unless stated otherwise.

RESULTS

Autoradiography of the gill arch exposed to [^{35}S]sulfate indicated that the branchial cartilage was the primary site of sulfate uptake (Fig. 1). Total sulfate uptake by ceratobranchial and epibranchial cartilage from the first 3 gill arches did not differ significantly (Table 1). There was a significant difference among individuals in this experiment ($P <$

Fig. 1. Autoradiograph of branchial cartilage exposed to 2 $\mu Ci \cdot ml^{-1}$ for 48 h. X-ray film was exposed for 4 d at −80°C. In freshly dissected preparations, cartilage (showing strong radiosulfate uptake above) is easily distinguished from bone by virtue of its transparency.

.0001, Wilcoxon test), indicating that a major source of variation is variation among individuals.

Hypophysectomy normally results in the cessation of feeding behavior in coho salmon. Hypophysectomized, sham-operated (starved), and intact (starved) coho salmon had lower condition factors (ratio of weight to length) than intact, fed fish (Table 2). Hepatosomatic index (HSI; liver weight as percent of body weight) of hypophysectomized coho salmon was intermediate between the fed and starved intact fish.

*TABLE 1. Sulfate uptake ($dpm \cdot \mu g^{-1}$) by cartilage of fed, 1 year-old coho salmon**

	Ceratobranchial	Epibranchial
Arch 1	5.53 ± 1.26	5.63 ± 1.49
Arch 2	6.01 ± 1.01	6.35 ± 1.25
Arch 3	4.95 ± 1.03	6.39 ± 1.39
Arch 1 (boiled)	0.19 ± 0.02	0.19 ± 0.01

*Values are mean ± SE, n = 6. There was no significant difference in total sulfate uptake between ceratobranchial and epibranchial or among the arches ($P > .5$, two-way ANOVA).

Hypophysectomized, sham-operated (starved), and intact (starved) coho salmon had similar levels of in vitro sulfate uptake (Fig. 2) in branchial cartilage. Sulfate uptake in these groups was approximately 30% of the levels in fed intact coho salmon. Starvation for shorter periods (5–10 d) was found to reduce sulfate uptake by at least 50% relative to fed controls.

In branchial cartilage from coho salmon starved for 5 d, in vitro exposure to recombinant bovine insulin-like growth factor I (rbIGF-I) at 1, 10, 100, and 1,000 $ng \cdot ml^{-1}$ caused a dose-dependent increase in sulfate uptake (Fig. 3). The maximum increase occurred at 1,000 $ng \cdot ml^{-1}$ rbIGF-I and resulted in levels almost 3-fold greater than controls. This increase is sufficient in magnitude to restore in vitro sulfate uptake to the levels of fed, intact fish (see Fig. 2).

Coho salmon insulin (1, 10, 100, and 1,000 $ng \cdot ml^{-1}$) resulted in an increase in in vitro sulfate uptake only at the highest dose used (Fig. 3), resulting in a 30% increase relative to controls. At these concentrations, insulin was approximately one order of magnitude less effective than rbIGF-I in stimulating in vitro sulfate uptake.

There was a slight decline in specific sulfate uptake relative to the control group when native chum salmon growth hormone (0.5 and 5 $\mu g \cdot ml^{-1}$) was added to the medium, although this effect was not statistically significant (Fig. 4). The addition of growth hormone did not significantly alter the ability of rbIGF-I (1 $\mu g \cdot ml^{-1}$) to stimulate sulfate uptake (Fig. 4). Triiodo-L-thyronine (T3; 0.5 and 5 $\mu g \cdot ml^{-1}$) had no effect on sulfate uptake, either in the absence or presence of rbIGF-I.

DISCUSSION

Results of this study indicate that mammalian IGF-I can have direct effects on sulfate uptake in cartilage from salmonid fishes. Native salmon insulin is much less effective in stimulating cartilage sulfate uptake, about one-tenth as effective as mammalian IGF-I. Growth hormone and triiodo-L-thyronine had no detectable direct effect during our 48-hour incubations. These results are consistent with previous findings in mammalian species in which IGF-I has been found to be an important regulator of cartilage metabolism and subsequent bone and whole-animal growth.

The results of this and other in vitro studies in teleosts support a somatomedin hypothesis for the regulation of cartilage metabolism in teleosts.

*TABLE 2. Length, weight, condition factor (100[weight · length $^{-3}$]) and hepatosomatic index (100 [liver weight · body weight $^{-1}$]) of juvenile coho salmon maintained at 14°C in fresh water**

	Length (cm)	Weight (g)	CF	HSI (%)
Fed, intact	16.3 ± 0.4	51 ± 4	1.16^{a} ± 0.04	1.41^{a} ± 0.07
Starved				
Hypophysectomized	16.8 ± 0.5	50 ± 4	1.03^{b} ± 0.02	1.04^{b} ± 0.07
Sham-operated	16.2 ± 0.4	42 ± 3	0.98^{b} ± 0.02	0.77^{c} ± 0.05
Intact	16.1 ± 0.3	41 ± 2	0.97^{b} ± 0.02	0.70^{c} ± 0.05
Wilcoxon test: P =	.62	.11	.0006	.0001

*Starved fish were deprived of food immediately after hypophysectomy or sham operation and for 22 d thereafter. Values are mean ± SE (n = 8 for each group). Values with different superscripts are significantly different (Wilcoxon test, P = .01, followed by Mann-Whitney test, $P < .01$).

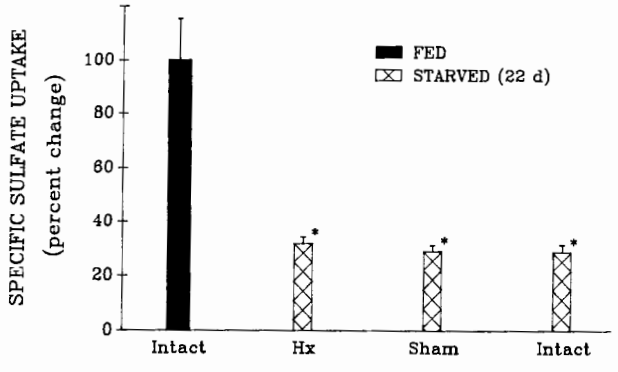

Fig. 2. Sulfate uptake in vitro by branchial cartilage (cerato- and epi-branchial) of coho salmon. Results are expressed as percent change from the mean value of the fed, intact group (n = 7–9 per group). *indicates a significant difference from the fed, intact group (Wilcoxon test followed by Mann-Whitney U-test, $P < .01$).

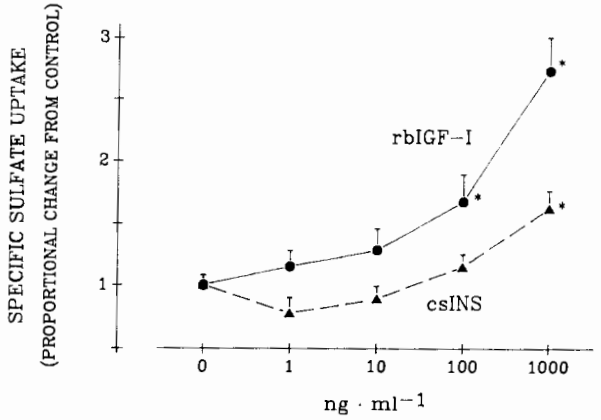

Fig. 3. Sulfate uptake in vitro by ceratobranchial cartilage from coho salmon starved for 5 d. Results are expressed as the proportional change (fold increase) from the mean value of the control group (no hormone added). *indicates a significant difference from the control group (Wilcoxon test followed by Mann-Whitney U-test, $P < .02$; n = 12–17 per group).

Hypophysectomy and starvation of eels, coho salmon, and long-jawed mudsuckers (*Gillichthys mirabilis*) reduces in vitro sulfate uptake in cartilage (Duan and Hirano, '90; present study; Gray and Kelley, '91). Growth hormone administration in vivo (Duan and Inui, '90a), but not in vitro (Duan and Inui, '90a; present study), increases cartilage sulfate uptake. GH injection results in increased liver IGF-I mRNA in coho salmon (Cao et al. '90) and increased plasma immunoreactivity to (mammalian) IGF-I in sea bream (*Sparus auratus;* Funkenstein et al., '90). Finally, mammalian IGF-I can stimulate in vitro sulfate uptake (Duan and Hirano, '90; present study; Gray and Kelley, '91), indicating that an endogenous IGF-I may also stimulate cartilage metabolism in vivo.

Starvation for 22 days resulted in a reduction of condition factor and a large decrease in in vitro sulfate uptake relative to fed fish (Table 2; Fig. 2). Hypophysectomy did not cause a further decrease in sulfate uptake from that seen in starved, intact fish. Interestingly, relative liver weight (HSI) of hypophysectomized fish was intermediate between starved and fed intact fish. This is probably the result of the absence of growth hormone (and perhaps other pituitary factors) which decrease liver lipid content and activate liver lipase activity in coho salmon (Sheridan, '86).

Administration of thyroid hormones increases whole-animal growth rates in teleosts (Donaldson et al., '79; Saunders et al., '85), although their effects have often been described as permissive. Barrington and Rawdon ('67) reported that thyroxine increased whole-animal growth rate and skeletal sulfate uptake in rainbow trout (*Oncorhynchus mykiss*). Komourdjian and Idler ('78) found that a combination of thyroxine and growth hormone stimulated sulfate uptake in bone of rainbow trout when liver tissue was present in the incubation medium, but not in the absence of liver tissue. Our results indicate that thyroid hormones do not directly affect sulfate uptake. There are several possible pathways for the indirect effects of thyroid hormones on cartilage metabolism and growth. Thyroid hormones directly stimulate proliferation of GH-producing cells (DeFesi et al., '84) and GH secretion (Casanova et al., '85) in mammals, and there is some evi-

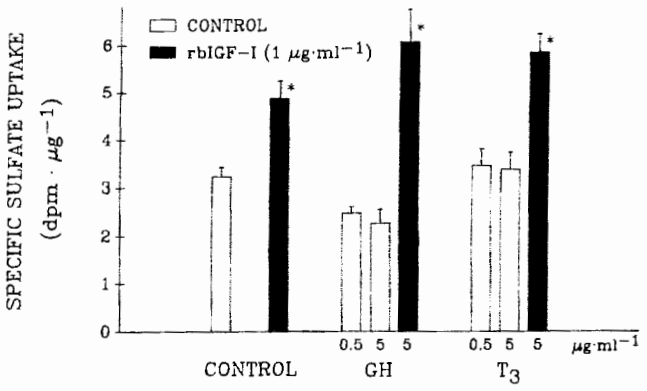

Fig. 4. Sulfate uptake in vitro by ceratobranchial cartilage from coho salmon starved for 5 d. Values are mean ± SE ($n \geq 8$ for each treatment). *indicates a significant difference from the control group (no hormone added; Wilcoxon test followed by Mann-Whitney U-test, $P < .01$).

dence for a direct effect of thyroid hormones on the release of growth hormone in salmonid fishes (Kelley, unpublished results). Another possible pathway for thyroid hormone action is the regulation of growth hormone receptors in liver, as occurs in mammals (Hochberg et al., '90). Such an action may explain the results of Komourdjian and Idler ('78) in which thyroxine was necessary for growth hormone to produce a "somatomedin-like factor" in incubations of liver slices.

Ceratobranchial and epibranchial cartilages of the first 3 gill arches had similar levels of sulfate uptake, indicating similar proteoglycan synthetic rates of these cartilage tissues. There were significant differences in sulfate uptake among individual coho salmon, which may reflect differences in individual growth rates. We have also found variations in responsiveness to IGF-I (e.g., between experiments presented in Figs. 3 and 4) which may reflect differences in the initial growth/endocrine status of the experimental animals. In addition, there are seasonal differences in sulfate uptake in coho salmon (unpublished results) which may reflect seasonal differences in growth rate or development. The precise relationship among cartilage sulfate uptake, growth rate, and their hormonal regulation in fishes has yet to be fully established.

Insulin has been shown to increase sulfate uptake (and have other somatomedin effects) in mammalian cartilage, though it is generally 1 to 2 orders of magnitude less effective than IGF-I (Froesch and Zapf, '85). These effects are generally believed to be the result of interation of insulin with the IGF-I receptor. In a similar fashion, IGF-I can cause hypoglycemia and other insulin effects in mammals when administered at high concentrations (Froesch and Zapf, '85). In the present study, recombinant bovine IGF-I can cause hypoglycemia and other insulin effects in mammals when administered at high concentrations (Froesch and Zapf, '85). In the present study, recombinant bovine IGF-I at a given concentration was 10 times more effective than native salmon insulin in stimulating cartilage sulfate uptake. The concentrations of insulin used in the present study encompass the circulating levels found in coho salmon (Plisetskaya et al., '91). Although teleost IGF-I is not currently available, it seems likely that it will be at least as effective as mammalian IGF-I (Cao et al., '90). It thus appears that IGF-I may be a more potent in vitro sulfation factor than is insulin in salmon, consistent with current mammalian growth models.

Duan and Hirano ('90) reported that mammalian IGF-I increased sulfate uptake in eel cartilage by up to 40-fold in a dose-dependent fashion. In the present study mammalian IGF-I increased sulfate uptake in cartilage from starved coho salmon to levels that were 3-fold higher than controls; such increases were sufficient to restore sulfate uptake to the levels seen in cartilage from intact, fed coho salmon. Similarly, Kelley and Gray ('91) have observed that mammalian IGF-I can stimulate a 4-fold increase in sulfate uptake by long-jawed mudsucker cartilage. Furthermore, we have found that under certain conditions, in vivo IGF-I administration can increase whole-animal growth rate of coho salmon (McCormick et al., '92). These results indicate that mammalian IGF-I has biological effects in teleosts, and that IGF-I may be an important regulator of cartilage metabolism and other aspects of growth in teleosts.

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LITERATURE CITED

Barrington, E.J.W., and B.B. Rawdon (1967) Influence of thyroxine upon the uptake of ^{35}S-labelled sulphate into the branchial skeleton of the rainbow trout (*Salmo gairdneri*). Gen. Comp. Endocrinol., *9:*116–128.

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

Casanova, J., R.P. Coop, L. Janocko, and H.H. Samuels (1985) 5′-flanking DNA of the rat growth hormone gene mediates regulated expression by thyroid hormone. J. Biol. Chem., *259:*6284–6291.

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

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

DeFesi, C.R., E.C. Fels, and M.I. Surks (1984) Triiodothryonine stimulates growth of cultured GC cells by action early in the G1 period. Endocrinology, *114:*293–295.

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

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

Duan, C., and T. Hirano (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.

Duan, C., and Y. Inui (1990a) Effects of recombinant eel growth hormone on the uptake of [^{35}S]sulfate by ceratobranchial cartilages of the Japanese eel, *Anguilla japonica*. Gen. Comp. Endocrinol., *79:*320–325.

Duan, C., and Y. Inui (1990b) Evidence for the presence of a somatomedin-like plasma factor(s) in the Japanese eel, *Anguilla japonica*. Gen. Comp. Endocrinol., *79:*326–331.

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

Funkenstein, B., A. Silbergeld, B. Cavari, and Z. Laron (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., J. J. Underwood, J. J. Van Wyk, and A.J. D'Ercole (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 K.M. Kelley (in press) Regulation of growth in the teleost, *Gillichthys mirabilis*: Role of growth hormone (GH), hepatic GH receptors, and insulin-like growth factor I. J. Endocrinol.

Hochberg, Z., T. Bick, and Z. Harel (1990) Alterations of human growth hormone binding by rat liver membranes during hypo- and hyperthyroidism. Endocrinology, *126:*325–329.

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

Ishii, T., and S. Kikuyama (1984) Uptake of [^{35}S] sulphate by *Xenopus* cartilage: the influence of growth hormone and prolactin. Zool. Sci., *1:*609–615.

Komourdjian, M.P., and D.R. Idler (1978) Hepatic mediation of hormonal and nutritional factors influencing the *in vitro* sulfur uptake by rainbow trout bone. Gen. Comp. Endocrinol., *36;*33–39.

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

McCormick, S.D., and H.A. Bern (1989) *In vitro* stimulation of Na+,K+- ATPase activity and ouabain binding by cortisol in coho salmon gill. Am. J. Physiol., *256:*R707–R715.

McCormick, S.D., K.M. Kelley, G. Young, R.S. Nishioka, and H.A. Bern (in press) Stimulation of coho salmon growth by insulin-like growth factor I. Gen. Comp. Endocrinol.

Nishioka, R.S. (1980) Hypophysectomy of tilapia (*Sarotherodon mossambicus*) through the orbit. Gen. Comp. Endocrinol., *40:*377–378.

Plisetskaya, E.M., L.I. Buchelli-Narvaez, R.W. Hardy, and W.W. Dickhoff (1991) Effects of injected and dietary arginine on plasma insulin levels and growth of pacific salmon and rainbow trout. Comp. Biochem. Physiol. [A], *98:*165–170.

Salmon, W.D., Jr., and W.H. Daughaday (1957) A hormonal controlled serum factor which stimulates sulfate uptake by cartilage in vitro. J. Lab. Clin. Med., *49:*825–836.

Saunders, R.L., S.D. McCormick, E.B. Henderson, J.G. Eales, and C.E. Johnston (1985) The effect of orally administered 3,5,3′-triiodo-L-thyronine on growth and salinity tolerance of Atlantic salmon (*Salmo salar*). Aquaculture, *45:*143–156.

Sheridan, M. (1986) Effects of thyroxine, cortisol, growth hormone, and prolactin on lipid metabolism of coho salmon, *Oncorhynchus kisutch*, during smoltification. Gen. Comp. Endocrinol., *64:*220–238.

Skyrud, T., O. Andersen, P. Alestrom, and K.M. Gautvik (1989) Effects of recombinant human growth hormone and insulin-like growth factor 1 on body growth and blood metabolites in brook trout (*Salvelinus fontinalis*). Gen. Comp. Endocrinol., *75:*247–255.

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