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INTRODUCTION

Pickford showed for the first time in 1948 that mammalian growth hormone was active in teleost fish such as Fundulus (*Fundulus heteroclitus*). Since this date, and until the eighties, the GH used in supplementation experiments in fish were all of bovine origin (Donaldson *et al.* 1979). Following this, scientists started treating animals with purified fish GH to estimate the biological activity of this preparation. The industrial production of massive amounts of recombinant fish GH by several different companies has enabled a large increase in the number of experiments using these homologous hormones.

These experiments demonstrated the pleïotropic effects of this hormone, which plays an important role in osmoregulation, reproduction and in both protein and lipid metabolism. However, it is mainly the effect on length and weight gain which has been the object of numerous experiments. The results show that the degree of response is very variable between the different experiments with exogenous GH; however, few researchers have analysed this variability.

Based on physiological data available and by analysing (multifactorial analysis) data collected from salmonids, the factors which may modify the response of fish to growth hormone treatment are listed. These variability factors are of two types:

- those linked to the hormonal preparation used and its mode of administration.
- those linked to the physiology of treated fish.

We have tried to situate the different levels of endocrine regulation which might be involved in this variability, based on a hypothetical scheme of the mechanisms involved.

I) HORMONE ACTIVITY AND ADMINISTRATION

a) Measuring techniques

In order to compare the effectiveness of different GH preparations on growth and on other functions, it is essential that the amounts administered are precisely known. Several techniques are available in order to measure this, each with its advantages and inconveniences.

Immunological assays (ELISA, RIA) give a very good quantification of the GH present. These have been developed in several teleost species such as carp, *Cyprinus carpio* (Cook *et al.* 1983), salmonids (Bolton *et al.* 1986, Wagner *et al.* 1986, Furuya *et al.* 1987, Le Bail *et al.* 1991, Farbridge and Leatherland 1991, Takahashi *et al.* 1991a), the Japanese eel, *Anguilla japonica* (Kishida and Hirano, 1988), tilapias (Hirano *et al.* personal communication, Ricordel *et al.* unpublished), and sea-bream, *Sparus aurata* (Le Bail *et al.* unpublished). The large majority of these assays use polyclonal antibodies. However, the immunological activity of the GH measured in this way is not generally identifiable with the biological activity, unless it has been specifically studied (Le Bail *et al.* 1991, Niu 1990, Smal personal communication). The use of monoclonal antibodies directed against the active site of the hormone molecule may lead to further research possibilities.

Several assays using homologous receptors are available for tilapia, *Oreochromis mossambicus* (Fryer 1979, Mori *et al.* in press), Coho salmon, *Oncorhynchus kisutch* (Gray *et al.* 1990) Rainbow trout, *Oncorhychus mykiss* (Yao *et al.* 1991) Japanese eel (Hirano 1991) and sea-bream (Perez-sanchez *et al.* 1991a).

Radioreceptor assay is directed against the binding site of the GH; it differs from radioimmunoassay which can be directed against epitopes dispersed over the whole molecule. The binding activity of the GH to its receptor is a necessary condition for obtaining biological activity. However, even if these two activities are very closein character, they cannot be confused, at least in theory, (see the antagonist case). Thus, this is not strictly speaking a biological test. However, the precision and the repeatibility of radioreceptorassay are superior to that of *in vivo* tests and by using a reference preparation it is possible to attribute and compare the binding activity of each preparation tested.

True biological tests or assays can be carried out either *in vivo* or *in vitro*, these two approaches being often complementary.

Certain authors have suggested an *in vitro* bioassay which is inspired from the rat tibia test. This involves measuring the quantity of radioactive sulphate incorporated into the ceratobranchial cartilages after stimulation by serum from animals treated *in*

vivo with GH (Ash 1977, Komourdjian and Idler 1978, Duan and Inui 1990). However, NIH researchers have now abandonned this complicated technique. To standardized the biological activity of their GH preparations, they now measure weight gain in hypox female rats injected with GH. A similar test, based on the reoccurance of growth after GH treatment in animals previously hypophysectomized, was the first *in vivo* biological test used in fish (Pickford 1954, Komourdjian *et al.* 1978, Kayes 1977). As hypophysectomy is difficult to perform in teleosts and as other means of characterizing the GH have become available, most authors have limited themselves to measuring length and and weight gain in entire animals (Table 1).

While this *in vivo* approach is necessary to demonstrate the real GH biological activity of a preparation, it presents three disadvantages:

- effects are difficult to quantify and reproduce because they depend on the physiological variability of the target animal as (genetic line, stage, stress, aquaculture conditions etc.). Moreover, diffusion and degradation of the injected hormone are difficult to control. In the majority of cases, these tests only give an indication as to the biological activity of the preparation.
- injected quantities are large (in general from 0.1 to 1.0mg/kg live weight).
- response time is at least approximately 10 days.

Simplification is thus necessary for making general use of reliable biological tests. Cell culture techniques are being increasingly used by scientists working on fish, and thus it is probable that in the near future a true biological *in vitro* test will be developed.

At the present time, radioreceptor assays are the most adapted tool for estimating biological activity of fish GH. However, there is no real standard test between laboratories. Thus, one should remain prudent when assessing the amounts of GH injected in experiments on fish (except in the case of NIH mammalian GH, as specific activities are well characterized).

b) Hormone quality

GH preparations, purified or recombinant, are rarely composed of only one biochemical entity. They may be composed of forms of different size (monomers, polymers), different charge or different secondary modifications (deaminated, phosphorylated, glycosylated etc.). These forms may exist in the natural state or be generated during manipulation. In mammals, these forms can have different biological activity (Charrier and Martal 1988). However, a recent study has demonstrated that, in trout, the different forms detected seem to have similar binding activity to the receptor (Niu 1990).

TARGET SPECIE GH USED	O. mykiss	O. kisutch	O. nerka	O. keta	O. rhodorus	S. salar	S. trutta	S. fontinalis	Esox americanus	Cyprinus carpio	Carassius auranus	Tilapia mossambica	Ophicephalus striatus	Gilichthys sp	Ictalurus melas	Fundulus heteroclitus	Anguilla anguilla	Anguilla rostrata	Anguilla japonica	AUTHORS
mammals							+ a						+ b			+ c				a) Swift 1954, b) Venugopalan 1967, c) Pickford *et al.* 1948
human	a + c d							+ c						+ b						a) Cotten 1987, b) Doneen 1976, c) Le Bail *et al.*, unpublished, d) Maclatchy *et al.* 1990, e) Skyrud *et al.* 1989
bovine	b + e k, l n, t	g + h m, q r	+ s						u	+ a	+ d p	+ c			+ j	+ o.		+ f	+ i	a) Adelman 1977, 1982, b) Chartier-Barbaduc 1959, c) Clarke *et al.* 1977, d) Cook *et al.* 1983, e) Danzman *et al.* 1990, f) Degani *et al.* 1985, g) Gill *et al.* 1985, h) Higgs *et al.* 1975, 1976, 1977, 1978, i) Inui *et al.* 1985a, j) Kaynes 1977, 1978, k) Leatherland *et al.* 1981, l) Le Bail *et al.*, unpublished, m) Markert *et al.* 1977, n) Niu 1991, o) Pickford *et al.* 1954, 1957, 1959, 1972, 1973, p) Prack *et al.* 1980, q) Richman III *et al.* 1987, r) Sheridan 1986, s) Swift 1954 , t) Weatherley *et al.* 1980, 1982, u) Weatherley *et al.* 1987
r bovine		+																		Down *et al.* 1988, 1989, Gill *et al.* 1985, Mc Lean *et al.* 1990
r bovine 21 K	+ b	+ a																		a) Down *et al.* 1989, b) Schulte *et al.* 1989
ovine	+ b d, f	+ e, n			+ l	+ a	+ k									+ h	+ e		+ i	a) Bœuf *et al.* 1990, b) Bolton *et al.* 1987, c) Björnsson *et al.* 1987, d) Collie *et al.* 1989, e) de Luze *et al.* 1984, 1987, f) Farbridje *et al.* 1988, g) Foster *et al.* 1991, h) Grau *et al.* 1979, i) Inui *et al.* 1985a et b, j) Kishida *et al.* 1987, k) Madsen 1990, l) Miwa *et al.* 1985, m) Wagner *et al.* 1985, n) Young 1988
porcine	+ a					+ b										+ c				a) Cheema *et al.* 1978, b) Komourdjian *et al.* 1976 a et b, c) Pickord *et al.* 1959
rat																	+			de luze *et al.* 1987
turkey, duck, ostrich																	+			de Luze *et al.* 1987
r chicken		+																		Gill *et al.* 1985
turtles																	+			de Luze *et al.* 1987
frog																	+			de Luze *et al.* 1987

TARGET SPECIE / GH USED	O. mykiss	O. kisutch	O. nerka	O. keta	O. rhodorus	S. salar	S. trutta	S. fontinalis	Esox americanus	Cyprinus carpio	Carassius auratus	Tilapia mossambica	Ophicephalus striatus	Gillichthys sp	Ictalurus melas	Fundulus heteroclitus	Anguilla anguilla	Anguilla rostrata	Anguilla japonica	AUTHORS
shark																+				Lewis *et al.* 1972
teleosteen																+				Pickford 1954
tilapia			+ a									+ a					+ b			a) Clarke *et al.* 1977, b) de Luze *et al.* 1984, 1987
bonite	+																			Noso *et al.* 1988
seriole	+																			Kawazoe *et al.* 1988
hake																+				Lewis *et al.* 1972
pollack																+				Pickford *et al.* 1959
cod	+																			Rand-Weaver *et al.* 1982
carp											+									Cook *et al.* 1983, Van der Kraak *et al.* 1990
eel	+																			Kishida *et al.* 1987
r eel																			+	Duan *et al.* 1990
trout	+																			Agellon *et al.* 1988, Danzman *et al.* 1990, Pector *et al.* 1990, Yao *et al.* unpublished
chum	+																			Bolton *et al.* 1987, Collie *et al.* 1989, Kawauchi *et al.* 1986, Komourdjian *et al.* 1979, Wagner *et al.* 1985
r chum	+ a, c	+ b		+ b							+ d									a) Kawauchi *et al.* 1986, b) Moriyama *et al.* 1990, c) Sekine *et al.* 1985, Singh *et al.* 1988
chinook	+																			Le Bail *et al.* 1989, Le Gac *et al.* in press.

TABLE 1: ACTIVITY OF GH FROM DIFFERENT ZOOLOGICAL ORIGINS ON FISH BIOASSAY.

Other than this polymorphism, the same form can be denatured to different degrees (cleavage, inadapted tertiary structure etc.) which renders it inactive. In the case of purified GH, the quality of the pituitaries (conservation etc.) or the choice of the techniques used (hydrophobic conditions, mechanic constraints, oxydation, enzymatic attack etc.) may be the cause of this inactivation.

Gray *et al.* (1990) have demonstrated that the binding activity of salmonid GH can vary from 1 to 25 depending on the method of preparation of the hormone. Even when the purification technique is standardized, the binding activity can vary from one preparation to another (Yao *et al.* 1991).

In the case of recombinant hormone produced by *E. coli*, the molecule did not undergo maturation and its tertiary structure does not conform to that of natural GH. A further step is thus necessary: renaturation. This step, which is in fact the key step in genetic engineering production, determines, for the greater part, the quality of recombinant hormones. Le Bail and Smal (unpublished) have found that, before renaturation, recombinant trout GH did not bind to liver GH receptors; while after adequate renaturation, this GH presented a binding activity similar to that of purified natural GH. Depending on the conditions of renaturation, recombinant GH can thus have different biological activity.

It should be noted that in the case of recombinant hormones produced by *E. coli*, contamination by proteins of bacterial origin could result in a toxic preparation which slows down growth.

c) Zoological specificities

Immunological similarities of GH from different zoological origins are variable. Immunodiffusion techniques (Hayashida 1970) or RIA developed for fish such as chum salmon (Wagner and Mckeown 1986, Bolton *et al.* 1986), chinook salmon (Le Bail *et al.* 1991), carp (Cook *et al.* 1983), eel (Kishida and Hirano 1988), sea bream (Le Bail *et al.* unpublished) and tilapia (Ricordel *et al.* unpublished), do not cross react with mammalian GH. Generally, immunological cross reactivity is total within the same family, partial within the same order and very weak between orders. However, in some cases, fish hormones from very different groups may be partially recognised by the antibody directed against the GH of the reference species (Le Bail *et al.* unpublished). These results confirm those obtained from GH cDNA, which shows that sequence homology between fish groups is partial (Kawauchi *et al.* 1990). So, injections of heterologous hormone risks producing an immune reaction in the treated animal which would diminish the effectiveness of the treatment, even to the extent of inducing auto-immunisation against its own GH that would block growth.

Table 2 summarises the effects of GH treatment in fish, using hormones from different zoological origins. It shows that mammalian GH are active in all the species tested. Further, all fish GH are active in all the fish species tested. In view of these results, it seems that vertebrate GH have similar biochemical structures which give them similar biological activities.

In order to analyse the relative biological activities of GH preparations, we carried out a multifactorial analysis on data obtained from GH supplementation experiments in Rainbow trout, described in the literature or obtained in our laboratory (Table 2). It shows (Figure 1a) that the greatest weight gains were obtained with salmonid GH and that other fish GH appear more active than mammalian GH. It should, however, be noted that this analysis brings together the greatest variability of results (Table 2) and does not take the doses of hormones used into account.

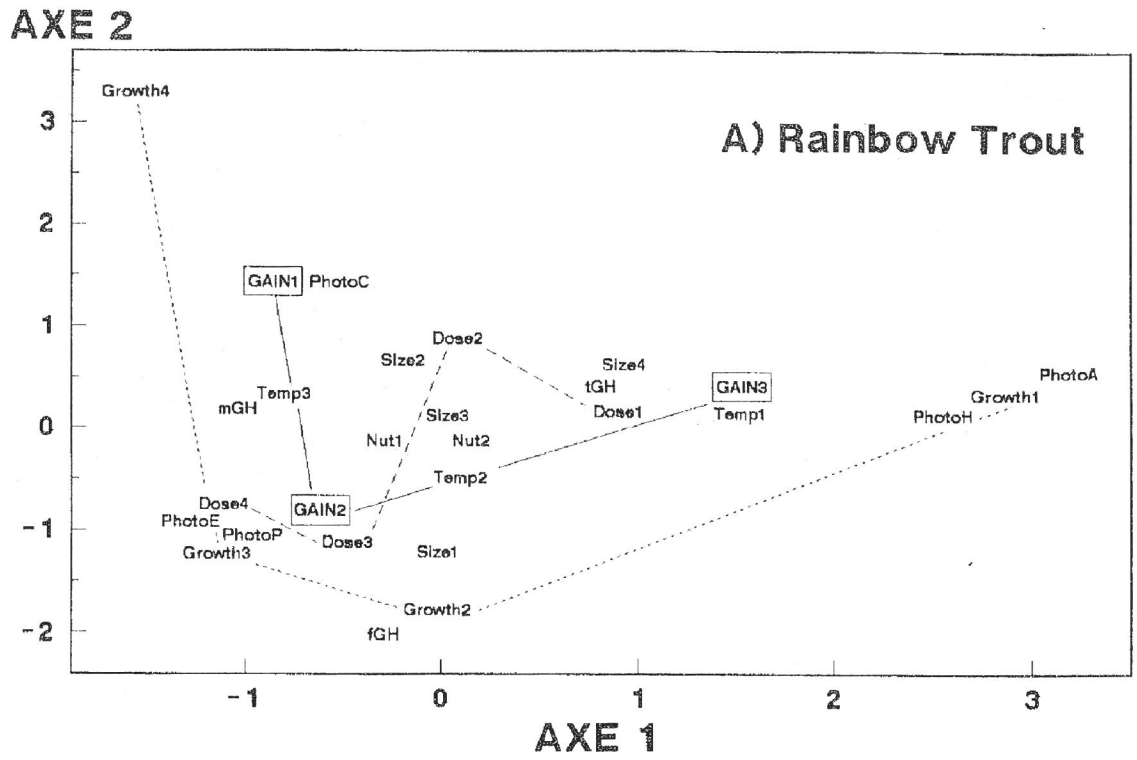
Comparison of relative potency of GH from different zoological origins during the same experiment are few. They show that in salmonids, human GH, bovine GH (natural and recombinant), ovine and chicken GH have comparable activities (Gill *et al.* 1985, Kishida *et al.* 1987, Le Bail *et al.* unpublished). Paradoxically, tilapia or salmon GH appear to be equipotent (Clarke *et al.* 1977, Wagner and McKeown 1985) or less active (Danzman *et al.* 1990) than mammalian GH tested in trout. These results, which are in apparent contradiction with those obtained in radioreceptor assays (see the following paragraph), could be explained by the injected doses which were too large and saturating or by denatured fish hormone preparation or by toxic fish hormone preparations, as may be the case in some experiments.

The biological activity on fish of vertebrate GH is confirmed by radioreceptor assays. All the GH tested were capable of binding to the salmonid receptor (Fryer 1979, Fryer and Bern 1979, Tarpey and Nicol 1985, Le Bail *et al.* 1989a, Niu 1990, Yao *et al.* 1991, Gray *et al.* 1990, Perez-Sanchez *et al.* 1991b), tilapia (Fryer 1979, Mori *et al.* in press), eel (Hirano 1991) and sea bream (Perez-Sanchez *et al.* 1991a).

The use of radioreceptor assays using hepatic membranes demonstrates the differences in activity between the hormones. These differences vary with the reference system used (hormone, receptor). With the exeption of ovine GH, that binds eel receptors with high affinity (Table 3), mammalian GH have only a few percent of the binding activity of homologous fish GH. Converesly, heterologous fish GH has an activity at least equal to 30 % of the homologous GH in all fish reference systems used. Tilapia GH (purified and recombinant) is a particular case, as it is practically inactive in eel or in salmonids. However, taken together, these binding study results confirm the results obtained in *in vivo* experiments, ie: as a rule, fish GH are generally more active than mammalian GH.

SPECIE	STRAIN	AGE	W	T°	PHO	SAL	NUT	GH	DOSE	ADM	TIME	CGR	GAIN	AUTORS
O. mykiss			25	12.5		0		Bovine	14	IP	14	0.860	1.035	Chartier-Baraduc 1957
O. mykiss			6	12	16	0	E	Bovine	20	IM	56	2.056	1.687	Weatherley et al 1980
O. mykiss			5	12	16	0	E	Bovine	10	IM	168	1.175	1.333	Weatherley et al 1982
O. mykiss			5	12	16	0	R	Bovine	10	IM	168	2.210	1.341	
O. mykiss			5	12	12	0	E	Ovine	2	IP	24	1.925	1.238	Wagner et al 1985
O. mykiss			5	12	12	0	E	Chum1	2	IP	24	1.925	1.202	
O. mykiss			5	12	12	0	E	Chum2	2	IP	24	1.925	1.202	
O. mykiss		0	10	9	12	0	E	Chum	0.1	IP	49	0.667	1.253	Sekine et al 1985
O. mykiss		0	10	9	12	0	E	rChum	0.1	IP	49	0.667	1.351	
O. mykiss			15	14	A	0	E	Chum	0.018	IP	24	0.330	1.113	Kawauchi et al 1986
O. mykiss			15	14	A	0	E	Chum	0.18	IP	24	0.330	1.434	
O. mykiss			15	14	A	0	E	Chum	0.1	IP	91	0.597	1.504	
O. mykiss			15	14	A	0	E	rChum	0.1	IP	91	0.597	1.542	
O. mykiss			13	15		0	R	Ovine	1.4	IP	25	1.723	1.150	Kishida et al 1987
O. mykiss			13	15		0	R	Eel	1.4	IP	25	1.723	1.165	
O. mykiss			13	15		0	R	Eel	1.4	IP	25	1.723	1.207	
O. mykiss	Kierk	0	20	13	P	0	E	Human	7.5	IP	56	1.316	1.390	Cotten 1987
O. mykiss	Kierk	0	20	13	P	0	E	Human	8.8	IMP	56	1.316	1.431	
O. mykiss			10	15.5	E	0	E	Bonite	0.1	IP	21	0.970	1.111	Noso et al 1988
O. mykiss			10	15.5	E	0	E	Bonite	1	IP	21	0.970	1.139	
O. mykiss	Normandale		110	10	12	0	E	Ovine	0.24	IP	28	1.629	1.064	Farbridje et al 1988
O. mykiss	Spring	0	1	15	12	0	E	rTrout	50	IMM	35	3.103	1.442	Agellon et al 1988
O. mykiss	Spring	0	1	15	12	0	E	rTrout	500	IMM	35	3.103	1.325	
O. mykiss	Spring	0	80	15	12	0	E	rTrout	0.2	IP	35	1.010	1.360	
O. mykiss	Spring	0	80	15	12	0	E	rTrout	1	IP	35	1.010	1.414	
O. mykiss			10	13	H	0	E	Cod	0.01	IP	20	1.013	1.134	Rand-Weaver et al 1989
O. mykiss			10	13	H	0	E	Cod	0.1	IP	20	1.013	1.169	
O. mykiss	Fraser	0	2	10	H	0	E	Bovine21	100	IMM	56	0.516	1.150	Schulte et al 1989
O. mykiss	Fraser	0	2	10	H	0	E	Bovine21	5	IP	56	0.783	1.710	
O. mykiss	Cornec	1	20	13	H	0	E	Chinook	0.1	IP	56	1.258	1.155	Le Bail et al 1989
O. mykiss	Reynold		40	17	12	0	E	Bovine	0.35	IP	33	1.677	1.160	Danzman et al 1990
O. mykiss	Reynold		40	17	12	0	E	rTrout	0.25	IP	33	1.677	1.050	
O. mykiss	Reynold	0	12	17	12	0	E	Bovine	1	IP	56	2.518	1.125	
O. mykiss	Reynold	0	12	17	12	0	E	rTrout	1	IP	56	2.518	0.970	
O. mykiss	Reynold	0	12	17	12	0	E	rTrout	0.1	IP	56	2.518	0.969	
O. mykiss	Reynold	0	12	17	12	0	R	Bovine	1	IP	56	2.349	0.982	
O. mykiss	Reynold	0	12	17	12	0	R	rTrout	1	IP	56	2.303	0.984	
O. mykiss	Reynold	0	12	17	12	0	R	rTrout	0.1	IP	56	2.303	0.939	
O. mykiss		0	50	11	P	0	R	Ovine	5	IMP	21	1.799	1.229	Foster et al 1991
O. mykiss	Cornec	1	140	15	P	0	E	Bovine	2	IP	28	1.327	1.144	Niu 1991
O. mykiss	Cornec	1	40	12	P	0	E	Bovine	2	IP	46	2.147	1.093	Le Bail et al unpublished
O. mykiss	Cornec	1	40	12	P	0	E	Human	2	IP	46	2.147	1.096	
O. mykiss	Mirwar		60	6.5	H	0	R	rTrout	0.1	IP	42	0.531	1.285	Smal et al unpublished
O. mykiss	Mirwar		60	6.5	H	0	R	rTrout	1	IP	42	0.531	1.401	
O. mykiss	Mirwar		60	7	H	0	R	rTrout	1	IP	42	0.605	1.420	
O. mykiss	Cornec	1	40	8	P	0	R	rTrout	0.5	IP	42	0.972	1.089	Yao et al unpublished
O. mykiss	Cornec	1	40	12	P	0	R	rTrout	0.5	IP	42	1.152	1.125	
O. mykiss	Cornec	1	40	16	P	0	R	rTrout	0.5	IP	42	1.159	1.127	
O. kisutch		1	10	10	12	0	E	Bovine	3.3	IMP	56	0.788	1.349	Higgs et al 1975
O. kisutch		1	10	10	12	0	E	Bovine	33	IMP	56	0.788	1.564	
O. kisutch		1	10	10	12	0	E	Bovine	20	IP	56	0.580	1.758	
O. kisutch		1	10	10	12	0	E	Bovine	100	IP	56	0.580	1.757	
O. kisutch		0	25	8	P	30	E	Bovine	10	IM	70	0.120	1.172	Higgs et al 1976
O. kisutch		1	100	8	P	30	E	Bovine	10	IM	70	0.284	1.183	
O. kisutch		0	8	10	12	0	E	Bovine	10	IP	84	0.651	4.085	
O. kisutch		0	8	10	12	0	E	Bovine	10	IM	84	0.525	4.915	
O. kisutch		0	8	10	12	0	E	Bovine	10	IM	84	0.423	4.056	
O. kisutch		0	8	10	12	0	E	Bovine	10	IM	84	0.513	3.467	
O. kisutch		0	8	10	12	0	E	Bovine	10	IP	84	0.588	4.005	
O. kisutch		0	8	10	12	0	E	Bovine	30	IP	84	0.588	4.176	
O. kisutch		1	12	10	12	0	E	Bovine	10	IM	59	0.850	2.032	Higgs et al 1977
O. kisutch		1	25	10	P	0	R	Bovine	10	IM	56	0.575	1.301	Markert et al 1977
O. kisutch		1	25	10	P	0	R	Bovine	10	IM	56	0.803	1.162	
O. kisutch		1	25	10	P	0	E	Bovine	10	IM	56	1.114	1.268	
O. kisutch		1	25	10	P	0	E	Bovine	10	IM	56	0.971	1.454	
O. kisutch		1	15	10	12	0	E	Bovine	0.1	IM	70	0.647	1.061	Higgs et al 1978
O. kisutch		1	15	10	12	0	E	Bovine	0.32	IM	70	0.647	1.202	
O. kisutch		1	15	10	12	0	E	Bovine	1	IM	70	0.647	1.271	
O. kisutch		1	15	10	12	0	E	Bovine	3.2	IM	70	0.647	1.364	
O. kisutch		1	15	10	12	0	E	Bovine	10	IM	70	0.647	1.658	
O. kisutch	Capilano	0	5	10	E	0	E	Bovine	1	IP	42	1.578	1.214	Gill et al 1985
O. kisutch	Capilano	0	5	10	E	0	E	Bovine	5	IP	42	1.578	1.329	
O. kisutch	Capilano	0	5	10	E	0	E	rBovine	1	IP	42	1.578	1.294	
O. kisutch	Capilano	0	5	10	E	0	E	rBovine	5	IP	42	1.578	1.400	
O. kisutch	Capilano	0	5	10	E	0	E	rChicken	1	IP	42	1.578	1.191	
O. kisutch	Capilano	0	5	10	E	0	E	rChicken	5	IP	42	1.578	1.386	
O. kisutch	Capilano	2	130	7.4	H	34	E	rBovine	0.5	IP	56	0.260	1.203	Down et al 1988
O. kisutch	Capilano	2	130	7.4	H	34	E	rBovine	5	IP	56	0.260	1.272	
O. kisutch	Capilano	2	130	7.4	H	34	E	rBovine	5	IP	56	0.260	1.182	
O. kisutch	Capilano	2	130	7.4	H	34	E	rBovine	0.5	IMP	56	0.260	1.189	
O. kisutch	Capilano	2	130	7.4	H	34	E	rBovine	5	IMP	56	0.260	1.112	
O. kisutch	Capilano	2	130	7.4	H	34	E	rBovine	0.5	PO	56	0.260	1.244	
O. kisutch	Capilano	0	3	11	E	0	E	rBovine	0.1	IP	56	2.109	1.034	Down et al 1988
O. kisutch	Capilano	0	3	11	E	0	E	rBovine	1	IP	56	2.109	1.112	
O. kisutch	Capilano	0	3	11	E	0	E	rBovin21	0.1	IP	56	2.109	1.137	
O. kisutch	Capilano	0	3	11	E	0	E	rBovin21	1	IP	56	2.109	1.316	
O. kisutch			4	12	E	0	E	rChum	3000	IMM	56	1.520	1.122	Moriyama et al 1990
O. kisutch			4	12	E	0	E	rChum	30000	IMM	56	1.520	1.207	
O. kisutch			30	10	E	0	E	rBovine	2.5	IP	49	1.659	1.551	McLean et al 1990
O. kisutch			30	10	E	0	E	rBovine	12.5	O	49	2.007	1.200	
O. kisutch			30	10	E	0	E	rBovine	12.5	O	49	2.007	1.293	
O. keta			2	7	H	0	E	rChum	5250	IMM	24	2.416	1.360	Moriyama et al 1990
O. keta			2	7	H	0	E	rChum	52500	IMM	24	2.416	1.500	
O. rhodorus	Kamazu	0	15	16	16	0	R	Ovine	2	IM	70	0.641	1.532	Miwa et al 1985
S. salar	St John	2	18	11.5	H	0	E	Porcine	3.5	IP	28	0.924	1.071	Komourdjian et al 1976
S. fontinalis		0	8	12	12	0	E	Human	10	IM	49	2.083	1.651	Skyrud et al 1988

TABLE 2: EFFECTS OF GH TREATMENT ON SALMONID WEIGHT INCREASE.
W(animal weight in grams), **T°**(breeding temperature), **PHO**(winter (**W**), spring(**Sp**), summer(**Su**), and autumn(**A**) photoperiod), **SAL**(salinity), **NUT**(food in excess(**E**) limited(**R**)), **GH**(GH origin), **DOSE**(dose of GH in μg/gram of wet weight), **ADM**(administration way, **IP**(intraperitoneal), **IM**(intramuscular), **IMM**(immersion), **PO**(osmotic pump), **O**(oral)), **CGR**(control growth rate), **GAIN**(treated growth rate/control growth rate).



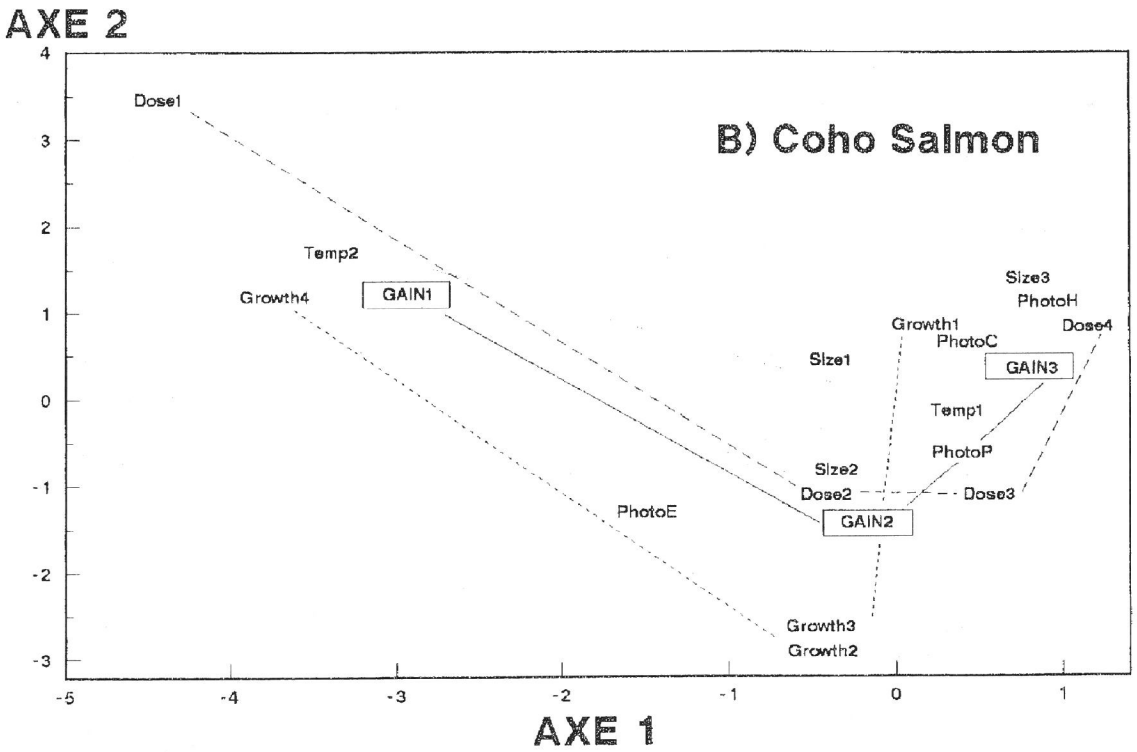


FIGURE 1: MULTIFACTORIAL ANALYSIS OF PARAMETERS WHICH MODULATE THE SALMONID RECEPTIVITY (GAIN) TO GH TREATMENT (Data from TABLE 2).
Numbers (1,2...) correspond to an increasing of the parameter value. **GAIN**(treated growth rate/control growth rate), **Growth**(control growth rate), **Size**(animal size at the beginning of the experiment), **Temp**(breeding temperature), **Photo**(winter(H), spring(P), summer(E) and autumn(A) photoperiod), **m**(mammalian), f(fish) and t(trout) **GH**, **Dose**(dose of the injected GH), **Nut**(food quantity).

TABLE 3: BINDING ACTIVITY TO LIVER GH RECEPTOR OF GH FROM DIFFERENT ZOOLOGICAL ORIGINS

human (Hum), bovine (Bov), ovine (Ov), sturgeon (St), tilapia (Til),
recombinant tilapia (rTil), Sea bream (Sb), Eel (Eel), recombinant Eel (rEel),
Chum salmon (Chum), recombinant Chum salmon (rChum),
Chinook salmon (Chinook), recombinant Trout (rTrout)

RECEPTOR	LABELLED HORMONE	BINDING POTENCIALITY AS PERCENT OF HOMOLOGOUS GH												AUTHORS
		Hum	Bov	Ov	St	Til	rTil	Sb	Eel	rEel	Chum	rChum	Chinook	rTrout	
Rabbit	Bovine	103	100		<0,4										Tarpey and Nicoll 1985
Tilapia	Tilapia		7	1-3		100				60		8			Fryer 1979
Sea Bream	Sea Bream	1	2	2			4	100						40	Perez et Le Bail unpublished
Eel	Eel			230		0,4			100	100		100			Hirano 1991
Coho	Chum	3	3			3					100	30	500		Gray *et al.* 1990
Trout	Chinook	3	3	3				50					100	100	Yao *et al.* 1990 Le Bail *et al.* unpublished

d) Mode of administration

Numerous experiments on exogenous GH application, particularly in salmonids (Table 2), have used different mode of administration. Repeated intraperitoneal or intramuscular injection and implantation methods are the most frequently used, as these need relatively small amounts of the hormone and the effectiveness is well documented. However, these methods are often incompatible with modern aquaculture techniques.

Other methods, such as oral and immersion administration have, thus, been explored. Several studies using these methods of administration have shown promising results (see review of McLean and Donaldson, 1990). However, absorption levels are low. Paradoxically, few data are available concerning the kinetics of diffusion or of absorption of the hormone during treatment. Plasma GH levels were monitered after injection or implantation in eel (Duan and Hirano 1991) and in trout (Le Bail *et al.* unpublished). Significantly higher levels than those of control animals are seen several hours after injection and up to 15 days after implantation. Plasma GH level evolution reveals, in both cases, an extremely high post-administration peak. A similar profile of plasmatic GH is observed in the case of intestinal absorption (Le Bail *et al.* 1989b). It is thus difficult to evaluate the minimal effective GH level as well as the minimal duration of exposure to this level. It is also clear that a significant part of the administered GH does not react. The amounts administered, generally expressed in μg of hormone per gram of live weight per week, cannot be compared between experiments except when using the same method of administration, otherways a large variability would be introduced.

Multifactorial analysis, carried out on the supplement experiments with GH in Coho salmon (Figure 1b), shows that the increase in growth is proportional to the injected dose of bovine GH. Three experiments carried out on Coho salmon (Higgs *et al.* 1978), sockeye salmon (Clarke *et al.* 1977) and carp (Adelman 1977) using 4 or 5 doses of bovine GH, show that the increase in growth is proportional to the logarithm of the dose (Donaldson *et al.* 1979). This dose effect has been confirmed by numerous other experiments which use bovine GH (Down *et al.* 1988, 1989), chicken GH (Gill *et al.* 1985) or fish GH (Noso *et al.* 1988, Rand-Weaver *et al.* 1989, Danzman *et al.* 1990, Moriyama *et al.* 1990, Smal *et al.* unpublished).

In trout, the lowest effective dose (0.01 μg/g/week) was obtained using cod GH (Rand-Weaver *et al.* 1989). Recombinant bovine GH is the most active of the mammalian GH (0.2 μg/g/week, Down et al. 1989). In certain cases, the strongest doses (>10 μg/g bGH/week) do not generate supplementary gain (Higgs *et al.* 1977) which implies that the totality of growth potential is expressed at weaker doses.

High doses may also have a depressive effect on trout growth (Farbridge and Leatherland 1988, Agellon *et al.* 1988) or provoke high mortality in cat fish (*Ictalurus melas*, Kayes 1977). These effects are as yet unexplained and could result from numerous factors, for exemple, toxic contaminants, insulin effect, metabolism rate too high for the environmental conditions etc.
So, the values of effective (or saturating) doses must be chosen, taking into account animal husbandry conditions and the receptiveness of the species for the GH used.

II) RECEPTIVENESS OF THE ANIMAL TO TREATMENT

a) Effect of stress

It is well documented that, in fish, a halt in growth can be provoked by a state of stress (Pickering, in press). Generally, studies have not taken into account the impact of stress during GH treatment even though less growth was seen in control animals which were injected with saline or had an implant without GH, than in controls which were simply anesthetized. It should be noted that control animals which were never handled were not taken into account in this type of experiment.
The experiments of Pickering *et al.* (1991), the most probing, show that the levels of plasma GH decrease several minutes after application of the stress factor (confinement in fish). After several hours, the levels increase (Cook and Peter 1984, Takahashi *et al.* 1991b). This increase probably reflects a decrease in the hepatic receptivity to GH and in consequence a decrease in the secretion of somatomedines (directly responsible for tissue growth) which are no longer able to control, negatively, the pituitary GH secretion. Under these conditions, weight growth would slow down or would even be blocked and exogenous GH may have no effect. However, this hypothesis remains to be proved.

b) Nutritional state

Multifactorial analysis applied to experiments carried out with trout gave no correlation between level of feeding and receptivity to exogenous GH (Figure 1a).
However, in a more specific experiment using Coho salmon, Markert *et al.* (1977) showed that an increase in calorie intake (quantity or quality of feed) brought about an increase in receptiveness to GH.
Taking these data into consideration we suggest that, in experiments where feed is limited inducing differences in food uptake between fish, the variability of the response

to GH treatment might be increased, more so as GH stimulates the food uptake (Donaldson *et al.* 1979, Jalabert *et al.* 1982).

c) Influence of growth rhythms

Multifactorial analysis does not show any effect of animal size on response to growth hormone. Results obtained in brown trout, *Salmo trutta*, show that the animals are able to respond as soon as they hatch (Vandeputte 1990), however, we do not know whether above a certain size, the degree of the GH response diminishes or is abrogated.

Generally, the duration of GH treatment in salmonids varies between 15 days and 2 months and it is not certain that GH treatment carried out for longer periods has the same effect. However, Weatherley and Gill (1987), using a GH treatment for 10 months on American pike (*Esox americanus*), observed a GH response throughout the experiment and obtained animals whose sizes were greater than record sizes of animals captured in the wild.

Fish growth follows annual (Marchand and Peter 1986) and monthly rhythms (Wagner and McKeown 1985, Cotten 1987) which can be seen with growth striations found in the bones and scales. A nycthemeral secretion of GH in the form of pulses (Le Bail *et al.* 1991), which are mainly synchronous (Marchand and Peter 1986, Takahashi *et al.* 1991b), has also been seen in fish. These variations in growth rate and GH secretion which are observed even when the breeding conditions are constant, probably influence the receptiveness to exogenous GH.

c) Influence of external factors

Several studies have demonstrated that long photoperiods stimulate growth in young Atlantic salmon (Stefanson *et al.* 1989a, 1989b, Saunders and Harmon 1990). This acceleration in growth is accompanied by an increase in pituitary GH content (Komourdjian *et al.* 1989). Multifactorial analysis shows that there is a link between low gain of growth rate due to GH treatment (controls had a high growth rate) and the summer photoperiod in Rainbow trout (Figure 1a) and in Coho salmon (Figure 1b). These experiments were carried out in natural conditions, the photoperiod and temperature were correlated and it is thus difficult to draw conclusions about which of these two factors is determinant in response to GH treatment.

The increase in growth rate due to GH is inversely proportional to temperature in Coho salmon and trout (multifactorial analysis). At very high temperatures (19°C), the effects of GH are suppressed (Danzman *et al.* 1990). In an experiment where the

effect of GH at different temperatures was studied, Yao *et al.* (unpublished) did not find this effect clearly. They showed however, that in controls, the level of circulating GH increased together with a decrease in hepatic receptiveness, when the temperature increased. This increase in plasma GH with temperature has also been observed in natural conditions (Barett and McKeown 1989). These results imply that salmonids raised at low temperatures are more suited to respond to a growth hormone treatment. When the temperature increase inhibits growth of the controls, GH treatment can once again be effective, as has been observed in carp (Adelman 1977).

The influence of water velocity has never been taken into account in studies on the effects of GH. However, a high water velocity stimulates growth in the arctic char, *Salvelinus alpinus* (Christiansen and Jobling 1990) and in the Rainbow trout (Le Bail *et al.* unpublished). This imposed physical exercise is accompanied by an increase in plasma GH (Barett and McKeown 1988a, 1988b, 1989). Response to exogenous GH of animals submitted to various strengths of current remains to be evaluated. These studies would have to take into account behaviour interactions between individuals and feeding conditions.

Throughout the passage from fresh water to sea water for euryhalin salmonids, the levels of GH (Sweeting *et al.* 1985, Collie *et al.* 1989, Boeuf *et al.* 1989, Rydevik *et al.* 1990) and IGF increase (Lindahl *et al.* 1985). This increase could explain the high growth rate observed in animals during their sea phase. Throughout this phase, the animals are still able to respond to GH treatment, as has been shown in Coho salmon (Down *et al.* 1988). These data are not sufficient to draw conclusions on whether the saline environment modifies receptivity to GH or not.

e) Species and Strain

The only species that have undergone a large number of experiments are Rainbow trout (39) and Coho salmon (43). Thus, a comparison can be made on their receptiveness to GH (Table 2), but the large variability in experimental conditions make it difficult to draw meaningful conclusions. It seems, however, that Coho salmon is more responsive to GH treatment than is trout, as 51 % of experiments carried out on the first species have a weight gain factor greater than 1.75 as compared to 27 % in trout.

The strains used in fish farms have very different geographical origins and have undergone a great deal of genetic selection pressure for growth. This selection which is unique to each fish culture, may act at different physiological levels in the mecanismes controlling growth (GH secretion, tissue receptiveness etc.). Thus, it

would be logical to observe subspecies which are more receptive than others, but no comparative experiments of this type have so far been carried out in fish to disprove this. In our laboratory, GH treatment separately carried out on two strains of trout induce systematically a lower gain of growth rate in one of these strains. This "strain" effect is probably responsible for the differences in response, observed in numerous experiments in fish.

III) ENDOCRINE MECHANISMS IMPLICATED

From the somewhat fragmentary results obtained from fish (see references Ch. II) and taking into account information aquired from mammals (Pell and Bates 1990, Ross and Buchanan 1990, Clemmons and Underwood, 1991), it is possible to draw up a regulation diagram to partially explain the "receptivity" of animals to GH (Figure 2). Stress diminishes the secretion of pituitary GH and probably increases the resistance of target GH tissues (Cook and Peter 1984, Pickering *et al.* 1991, Takahashi *et al.* 1991).

Hypotheses put forward to explain the effects of nutrition are drawn from results obtained from starved fish. We found that starvation decreases the apparent number of GH receptors (Yao and Le Bail, unpublished) in the target tissues, which probably induces GH resistance. Plasma levels of IGF decrease (Komourdjian and Idler 1978, Yao and Le Bail unpublished), which probably has the effect of increasing the plasma levels of GH (Barett and McKeown 1988a, Sumpter *et al.* 1991a, 1991b), as an inhibitory action of IGF on the pituitary GH secretion has been demonstrated (Perez-Sanchez *et al.* 1991c). In these circumstances, exogenous GH is without effect on growth.

Increase in receptivity to GH in salmonids raised at low temperatures is explained by the lower levels of circulating GH. The number of free hepatic GH receptors also increase (Yao and Le Bail, unpublished), which suggests a better potential receptivity of animals to exogenous GH treatment. However, the number of free receptors is inversely proportional to the level of circulating GH (Le Bail *et al.* unpublished), which might demonstrate the amount of receptor occupation and not the total number of receptors (Sakamoto and Hirano, in press). So, we do not know yet if temperature could influence the total number of GH receptors.

The affinity of the GH receptors, which is not modified by variations of the water temperature (Yao and Le Bail, unpublished) or by variations in the salinity (Sakamoto and Hirano, in press), would have no influence on fish receptivity to GH treatment.

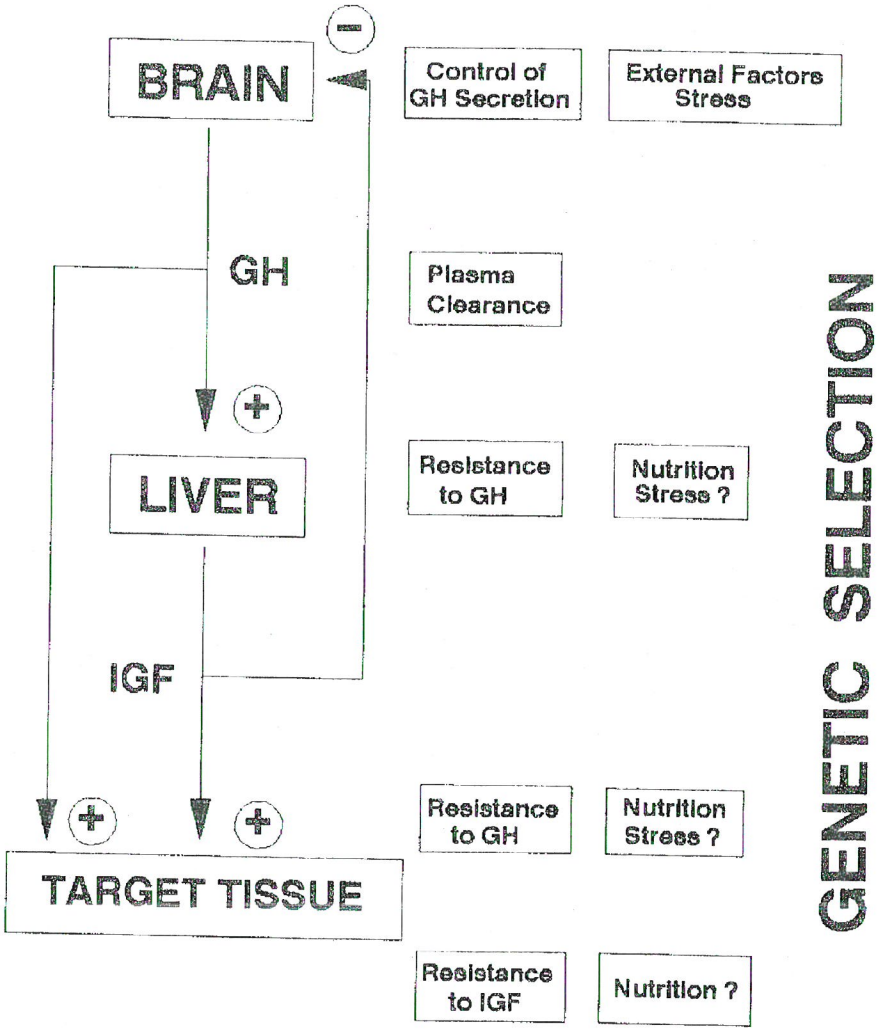


FIGURE 2: DIAGRAM OF ENDOCRINE MECHANISMS IMPLICATED IN THE RECEPTIVITY TO GH TREATMENT.

Modifications in GH plasma clearance rate could also be a regulatory factor. As few studies have been carried out in this field, no conclusions can be drawn (Le Bail et al., 1989; Le Bail and Perez-Sanchez, unpublished). However, Duan and Hirano (1991) did not observe any significant modifications in clearance between eels raised in fresh-water or in salt-water.

From the information available, it is reasonable to assume that temperature, and perhaps other external factors, act principally on pituitary GH secretion.

CONCLUSION

GH from all zoological origins are active in fish. The biological activity of fish GH, determined using fish radioreceptor assays, is higher than that of mammalian GH. However, the quality of fish GH preparations is very variable. The amount of GH injected or implanted influences the plasma levels attained but also the length of time necessary for these to return to normal levels. Thus, the effect measured results of the combination of time effect and dose effect. This lack of control over the methods of administration explains that, when different GH are tested *in vivo* on the acceleration of weight growth, the differences are not as clear as when testing them in a radioreceptor assay. A standardization of *in vivo* and *in vitro* bioassays should be developed in order to make future experimental results coherent and comparable.

The multifactorial analysis (Figure 1) demonstrates a strong negative correlation between the growth increment in treated animals and the growth rate of control animals. This phenomenon is observed in trout and in Coho salmon. The growth rate reflects and integrates the factors which modulate growth physiology, such as environmental factors. This implies that the more the breeding conditions (other than food) are unfavourable to growth, the greater the response is to GH treatment.

The same reasoning could apply concerning the genetic characteristics of animals. If a strain has a high growth rate, it should respond less to GH (especially if this selection increases the endogenous GH secretion). This situation might correspond to that in mammals and in birds where GH treatment has little or no effect on growth rate of farmed subspecies, which have undergone large selection pressures to increase their growth rates. Only dwarf animals are receptive to exogenous GH.

Fish would thus have similar growth control mechanisms to mammals. The spectacular differences in receptiveness to GH treatment observed between mammals and fish might be explained for the greater part by:

- genetic selection which has not yet used all the growth potential linked to GH secretion in fish.

- rearing temperature which is often far from the optimal growth temperature of fish, which, it should be remembered, are poikilothermic animals.

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