A PRELIMINARY EXPERIMENT ON THE GROWTH PROMOTING
EFFECT OF GROWTH HORMONE WITH THYROID-
STIMULATING HORMONE AND PROLACTIN TO THE YOUNG
RAINBOW-TROUT (*Salmo irideus*)

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Introduction

In the previous work³⁾, the author reported the extraction of bovine growth hormone after A. E. WILHELM's method and its growth promoting effect to the young carps. Recently, G. E. PICKFORD⁹⁾ suggests the co-operative growth promoting effect of growth hormone with the other anterior pituitary hormones.

Here, the results of a preliminary combination experiment of bovine growth hormone (GH) with thyroid-stimulating hormone (TSH) and prolactin to the young rainbow-trouts are presented.

Material and Methods

Two hundreds of young rainbow-trouts hatched out in the early spring of 1963 and reared in the Okutama Branch of the Tokyo Fisheries Experimental Station, were transported by car to the Freshwater Fisheries Research Laboratory at Hino on August 1, 1963, precultured with bovine liver for five days, and then were applied to experiment from August 5.

Eight groups, each comprising three rainbow-trouts, were set in the eight glass tanks of 37 cm. × 20 cm. widths and 25 cm. in height, and in each tank, each of three fish was separated with net and reared in the running water of 16°C. containing about 10 γ /L phosphate and 200 γ /L nitrogen in the rate of 360 litres an hour.

The fish were fed with pellets of which the contents were as follows: crude protein 40 %, crude fat 2 %, crude cellulose 5 %, crude ash 14 %, Ca CO₃ 0.1 % and the remainder carbohydrates.

Ten grains of this pellet (0.5 g.) were supplied to each rainbow-trout in the morning and the same in the afternoon. This ration does not show the values eaten by rainbow-trout actually, but does more, as some remained always in the bottom of the tank. The eight different combinations of injections were applied to the eight groups, respectively (Table 1). Injections were made intramuscularly between dorsal fin and lateral line to urethane-narcotized rainbow-trout which was fully starved for a day before the injection. Injections were made in every fourth day, being six times

Table 1. Doses of three anterior pituitary hormones, **GH**, **TSH** and Prolactin per injection to each of young rainbow-trouts (*Salmo irideus*).

Group number	Number of fish	GH*	TSH**	Prolactin***	0.7 % saline solution
1	3	—	—	—	0.2 cc.
2	"	70r	—	—	"
3	"	70r	0.02 IU.	—	"
4	"	70r	—	1 IU.	"
5	"	70r	0.02 IU.	1 IU.	"
6	"	—	0.02 IU.	—	"
7	"	—	—	1 IU.	"
8	"	—	0.02 IU.	1 IU.	"

* **GH** potency: Equi-potent with USP Reference Standard (1 unit/mg.).

** **TSH** used here has a trade name of Thytropar from Armour Phorm. Co., U.S.A.

*** Prolactin used here is the lactogenic hormone from Teikoku-Zoki Co. Japan.

in total. After 27 days, the fish were killed, weighed and measured in length, and some researches were worked out with blood as remarks.

Results

All the results are shown in Table 2.

As to the 1st group to which saline solution was applied, all three individuals were safe and showed 36% gain in mean body weight. As to the 2nd group, to which saline solution comprising **GH** was applied, the healthy one showed 108% gain in body weight while the other two suffered from some exophthalmic disease*. As to the 3rd group, to which saline solution comprising **GH** and **TSH** was applied, also two of them suffered from the exophthalmos and the healthy one showed 96% gain in body weight. As to the 4th group, to which **GH** and prolactin was applied, also two suffered from exophthalmos and the healthy one showed 53% body weight gain. As to the 5th group, to which saline solution comprising **GH**, **TSH** and prolactin was applied, one suffered from exophthalmos while the other two showed 46% gain in mean body weight.

As to the 6th group, to which saline solution comprising **TSH** was applied, all three were safe and showed 44% gain in mean body weight. As to 7th group, to which saline solution comprising prolactin was applied, all three were safe and showed 37% gain in mean body weight. As to 8th group, to which saline solution comprising **TSH** and prolactin was applied, all three were safe and showed 36% gain in mean body weight.

Discussions

The scarcity of the number of experimental rainbow-trouts, as three in each group,

* Details with this fatal exophthalmic disease are to be presented in the succeeding paper.

made this experiment of a preliminary one. The scarcity was due to the fact that they had been reared separately in such a space mentioned-above to avoid their quarrels and competitions.

Table 2. The results of the eight rainbow-trout groups for **GH**, **TSH** and prolactin injection experiments (reared 27 days from Aug. 12 to Sept. 7, 1963).

Naked numerals in length and weight show the values at the beginning and those in parentheses at the end of the experiments.

Group	Fork length (%)	Body weight (g.)	Rate of wt. gain (%)	Reticulo-cyte cell (%)	White blood cell count	Remarks
Control	8.3(9.7)	7.0(10.2)	46	9.3	34	
	8.4(9.3)	7.6(9.6)	26	8.2	26	
	8.2(9.3)	6.8(9.2)	35	8.4	32	
GH	8.7(—)	7.9(—)	—	—	—	Exophthalmos
	8.8(11.5)	8.5(17.7)	108	9.0	36	
	8.5(10.0)	7.8(12.0)	54	11.1	26	Exophthalmos
GH and TSH	8.4(—)	6.7(—)	—	—	—	Exophthalmos
	8.5(—)	7.0(—)	—	—	—	Exophthalmos
	8.7(11.1)	7.6(14.9)	96	11.0	24	
GH and Prolactin	9.1(9.5)	8.5(9.2)	8	—	—	Exophthalmos
	8.7(—)	8.0(—)	—	—	—	Exophthalmos
	9.2(10.3)	8.9(13.6)	53	15.6	30	
GH, TSH and Prolactin	8.6(—)	7.2(—)	—	—	—	Exophthalmos
	8.8(10.1)	8.6(11.5)	34	10.1	58	
	8.8(9.8)	7.7(12.1)	57	13.9	44	
TSH	8.2(9.0)	7.1(10.0)	41	5.6	26	
	8.6(9.7)	8.3(11.9)	43	7.9	29	
	8.2(9.3)	7.2(10.7)	49	4.6	24	
Prolactin	9.1(9.9)	4.0(11.4)	26	5.4	28	
	8.4(9.4)	7.2(10.5)	46	8.3	48	
	9.1(10.2)	9.3(13.0)	40	5.4	28	
TSH and Prolactin	8.5(8.9)	7.4(8.8)	19	5.8	28	
	8.3(9.3)	6.7(9.1)	36	6.9	25	
	9.0(10.2)	9.3(14.3)	54	4.0	26	

Also, the unexpected death by an exophthalmic disease in four groups to which **GH** was applied with other hormones, made the analysis of the result more difficult. Yet, some foods for further study might be drawn from this results. In this paper, the results of fishes excluding those, dead or alive, suffered from exophthalmic disease, are discussed. Then, two individuals were conspicuous in body weight gain, the one showed 108% belonging to the 2nd group injected with **GH** and the other did 96% belonging to the 3rd group injected with **GH** and **TSH**, compared with 36% mean

body weight gain in control. The other groups may be said to have not shown any obvious gain, showing 53 %, 45 %, 44 %, 37 % and 36 %, respectively, compared to 36 % in control.

The chemical analysis of the total body was worked out with the two of control and the two which showed obvious body weight gains to know what constituted the body weight gain. As shown in Table 3, the ratios of crude protein to crude ash in **GH**-injected and **GH** and **TSH**-injected ones were much larger as 11 to 12 than those in control of 7 to 7.5. This results coincide with the protein-anabolic agent of **GH**.

Table 3. The results of chemical analysis of the total body of **GH**-, **GH** and **TSH**-injected rainbow-trouts in comparison with those of control.
(Numerals were shown in comparison with ash.)

Group	Crude ash	Crude fat	Crude protein	Remarks
Control { 1 2	1	2.17	6.89	Fixed with 10% formol
	1	2.98	7.53	"
GH	1	2.77	10.90	"
GH and TSH	1	3.14	12.07	"

Though too few in numbers of experimented fish, it might be anticipated that **GH** independently or with **TSH** have some growth promoting effect and would be of some meaning to study further in these series in a larger number, in a longer period and in the various hormone doses.

Besides, as to the blood research, the large values of reticulocyte* cell counts in four groups to which **GH** was injected with or without other hormones coincide with the anaemia in the gill observation and might have some relation with exophthalmic disease which occurred also in the same four groups to which **GH** was applied.

Any conspicuous phenomenon was not observed in white blood cell count and relative number of different white blood cells as shown in Table 3.

Summary

GH, **TSH** and prolactin were injected intra-muscularly in their various combination to the young rainbow-trouts in summer of 1963, preliminarily. Their body weight gains and some other characters were measured and compared with those of control after rearing for 27 days.

1) Two rainbow-trouts, the one injected with 70 γ of **GH** in every fourth day and the other done with that and 0.02 I. U. of **TSH**, showed some conspicuous body weight gain as 108 % and 96 %, respectively, compared with 36 % in control.

2) These weight gains may be mainly attributed to the gain in protein judged

* Reticulocyte here contains polychromatic erythrocyte and basophilic erythrocyte with the blood stained by WRIGHT's solution.

from the chemical analysis, as expected from the protein anabolic agent of **GH**.

3) Some exophthalmic disease occurred in the **GH**- injected groups to which the large values of reticulocyte cell counts were also observed.

The further experiments of these series should be worked out in a larger number, in a larger period and with various hormone doses.

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