

A Quantitative Study of Thyroxin—Thyrotropic Hormone Interaction¹

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The problem of finding a suitable biological assay for thyroid stimulating hormone (TSH) in body fluids is complicated by the report that endogenous thyroid hormone has an inhibitory effect on TSH (Aron, 1930; Loeb, Bassett and Friedman, 1930; Loeser, 1934; Cortell and Rawson, 1944). D'Angelo and Gordon (1950) report, however, that the stasis tadpole technique for assay may be used to detect simultaneously both thyroxin and TSH, since TSH increases the thyroid cell height, while thyroxin causes an accelerated hind limb growth. The two effects reportedly are additive, indicating that one is not affected by the other. Recently, however, Steinmetz (1951) has shown by factorial analysis, using the original data of D'Angelo and Gordon, that there is interaction with inhibition between TSH and thyroxin in the stasis tadpole. The present report is an attempt to study quantitatively the interaction between TSH and thyroxin.

Methods

Day old White Leghorn male chicks were used. They were kept in groups of 20-25 in compartments of standard chick "Pullman" boxes without food or water for the duration of the experiment. Different series were distinguished by coloring the head and back with colored dyes. Ten animals were allotted to each series. The chicks were injected twice a day for 3 days in the cervical region with varying doses of TSH² or thyroxin³ in ½ ml. amounts, or with combinations of the two hormones. Controls were injected with ½ ml. of physiological saline. At the end of the 3 day injection period, the animals were sacrificed, and the thyroids were removed quickly, cleaned of non-thyroidal tissue, and weighed on a torsion balance to the nearest 0.02 mg. The glands were then fixed in 10% formalin for sectioning. Five μ sections were cut from five of the glands of each series and the tissues were stained with Azan stain. Ten sections scattered throughout the gland were chosen at random for reading, and one cell from a distinct follicle at the edge of each section, and one cell from the middle of each section was measured with a filar micrometer at a magnification of 1470 times.

The doses of thyroxin used (1, 5, 10 μ gm) were chosen because Odell (1952) reported that 10.3 μ gm equivalents of D-L thyroxin were secreted per day by 40-day-old White Leghorn cockerels. The thyroxin used in these experiments was L-thyroxin, which is the physiologically active isomer of the thyroid gland, and thus, is about twice as active as the mixture. The 5 μ gm dosage should, therefore, be more than sufficient for the chicks.

1. Contribution No. 541 from the Department of Zoology, Indiana University.
2. USP Thyrotropin reference standard. Each mg. represents 0.05 USP Thyrotropin units.
3. Squibb crystalline D-thyroxin.

Results

Analysis of variance was the statistical treatment used to analyze the interactions between TSH and thyroxin. The cell heights in the various series are presented in table I. The acinar cells of the thyroids of the control series averaged 1.75μ in height. The cells of the thyroids which received 0.01 USP units of TSH averaged 1.80μ in height. This was a non-significant increase. The chicks which received 0.025 and 0.05 USP units of TSH had cell height averages of 2.08μ and 5.01μ respectively, and both increases were significant at the 1% level, as determined by "t" test. The thyroids of the birds receiving thyroxin averaged 1.96, 1.57, and 1.58μ respectively for dosages of 1, 5, and $10 \mu\text{gms}$ of thyroxin. The $1 \mu\text{gm}$ dose caused cell height increases which were significantly greater than the controls, while the 5 and $10 \mu\text{gm}$ doses caused significant cell height decreases.

TABLE I. Thyroid cell heights of chicks treated with TSH and thyroxin, alone and in combination.⁴

		TSH - USP units				Sum	Mean
		0	0.01	0.025	0.05		
Thyroxin - $\mu\text{gms.}$	0	1950 1.75 μ	2004 1.80 μ	2313 2.08 μ	5569 5.01 μ	11836	29.59
	1	2176 1.96 μ	1878 1.69 μ	4506 4.06 μ	5469 4.92 μ	14029	35.07
	5	1738 1.57 μ	1697 1.53 μ	4361 3.92 μ	5955 5.36 μ	13751	34.38
	10	1757 1.58 μ	1787 1.60 μ	3739 3.36 μ	5072 4.56 μ	12355	30.89
Sum		7621	7366	14919	22065	51971	
Mean		19.05	18.42	37.30	55.16		129.93

4. The upper figure is the sum of the readings for each group. These figures were not converted to microns for greater ease in handling calculations. The lower figure in each group is the mean cell height in microns.

TABLE II. Analysis of Variance. Thyroxin - TSH data.

Source of Variation	d.f.	Sum of Squares	Mean Square
Thyroxin	3	8483.88	2827.96**
TSH	3	366330.43	122110.14**
Interaction	9	27316.41	3035.16**
Error	1584	57932.75	36.57
Total	1599	460063.47	

The analysis of variance in table II shows that thyroxin and TSH both have a significant effect on the bird's thyroids, and also that there is an interaction between thyroxin and TSH. This is most striking in the third column where 0.025 units of TSH was administered. Here the thyroxin appears to have a synergistic effect in combination with TSH as shown by the increased cell heights of the thyroids receiving the combined hormones over those thyroids which received TSH alone. All other doses of TSH plus thyroxin show a decrease in cell height as the thyroxin is increased, with the one exception of the 0.05 units of TSH plus 5 μ gm of thyroxin series where the cell height is again increased significantly over that of the 0.05 unit TSH series cell heights.

Discussion

Fugo (1940) reports that the chick anterior pituitary becomes active during late embryonic stages, and that TSH is being released at the time of hatching. The amount of TSH must be very small, however, since sections of normal 4-day-old chick thyroids show follicles which are distended with colloid, and have squamous epithelial cells with darkly staining pycnotic nuclei. Both conditions are indicative of an inactive thyroid. Breneman (1941), and Payne (1942) report that the day-old chick, under starvation conditions, has an anterior pituitary which is nearly or completely inactive. We are thus using the equivalent of a hypophysectomized animal for a biological assay. Cortell and Rawson report no change in thyroid cell height in hypophysectomized rats treated with thyroxin, but since thyroxin caused a decrease in the cell height in chicks, it appears likely that the day-old assay birds are releasing minute quantities of TSH. This amount, however, is insufficient to cause much activation of the thyroid gland.

The TSH injected birds (Row 1) show no significant increase in cell height between 0 and 0.01 TSH units, whereas there is a significant increase in cell height when 0.025 units are given and a very sharp increase in cell height between 0.025 and 0.05 units of TSH. It appears that the threshold level for TSH lies between 0.01 and 0.025 units. A series of graded doses of TSH between these levels should be run to determine the amount of TSH which serves as the threshold level.

Two important points stand out in the thyroxin injected series. First, the injection of thyroxin at the 1 μ gm level causes an increased cell height. This is contrary to the conclusions of Cortell and Rawson, and others, that administration of thyroxin causes a decrease in cell height of the thyroid. The higher doses of thyroxin, however, give the expected decrease in cell height. The second point to be noted is that there is a synergistic action between 0.025 units of TSH and the various dosages of thyroxin.

There are two possible explanations for these results. (1). Since the chicks are without food or water from the time of hatching until they are sacrificed at 4 days, the general body metabolism is undoubtedly decreasing. Injection of a low dosage of thyroxin may cause an increase in the cellular metabolism of the thyroid without producing inhibition. TSH, therefore, may be more effective and may stimulate the thyroid, thus producing greater cell height. Measurement of cellular metabolism of the

thyroids would help determine the correctness of this hypothesis. (2). Cellular metabolism of the pituitary also may be increased by injection of thyroxin. With an increased metabolism, the pituitary may release more TSH, which in turn would produce greater stimulation of the thyroid. If this hypothesis is correct, one might also expect to find an increase in other pituitary hormones. This could be checked by comparing weights of other endocrine organs, and by direct assay of the pituitaries.

Summary

Day-old White Leghorn male chicks were injected with varying doses of thyroxin or TSH, or combinations of the two. Cell height measurements of the thyroids showed that 1 μ gm of thyroxin caused increased cell height instead of the expected decrease. It was also found that 0.025 USP units of TSH, in combination with thyroxin caused cell height increases, indicating synergistic action between the hormones. Other combinations of the hormones caused decreased cell heights. Two possible explanations are given for these results. (1). Increased thyroid cellular metabolism, or (2). increased pituitary cellular metabolism, with increased TSH production.

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