

## EFFECT OF THYROIDECTOMY ON PITUITARY LUTEINIZING HORMONE (LH) CONCENTRATION IN FEMALE RATS

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### ABSTRACT

W. Chia-Mo Wan and Ji-Chuu Hwang (1973) *Effect of Thyroidectomy on Pituitary Luteinizing Hormone (LH) Concentration in Female Rats*. Bull. Inst. Zool., Academia Sinica 12(1): 39-44. With sufficient calcium supplementary in diet, the thyro-parathyroidectomized rats showed a decline in pituitary LH concentration, prolonged estrous cycle, and decreased ovarian weight. After two weeks thyroxine treatment, the pituitary LH concentration and ovarian weight of these rats were restored. Possible regulatory mechanisms have been discussed.

Abnormality in mammal reproduction induced by hypothyroidism has been reported repeatedly<sup>(2,3,8,11,13,14,19,22,25)</sup>. Only a few works on changes in the pituitary gonadotrophin have been found<sup>(5,6,15,26)</sup>. Ascertaining the minimal quantity of pituitary tissue from thyroidectomized donors to initiate the repairment of ovarian interstitial tissue of hypophysectomized recipients, Contopoulos *et al.*<sup>(5,6)</sup> demonstrated a decline of pituitary interstitial cell stimulating hormone (ICSH) in rats after thyroidectomy. However, changes of concentration of ICSH in the pituitaries of the thyroidectomized rats have not been reported. In order to determine the changes of concentration of pituitary luteinizing hormone (LH) in thyroidectomized rats the ovarian ascorbic acid depletion (OAAD) method was employed in this experiment.

### MATERIALS AND METHODS

Eighty-90 days old virgin female rats of the

Sprague-Dawley strain from this laboratory were used as subjects. The rats were housed in a room with regulated temperature ( $23 \pm 1^\circ\text{C}$ ) and light (6 AM-6 PM). Vaginal smears were examined daily before 10 AM. Rats that demonstrated at least two 4-day regular cycles were chosen for this investigation.

During estrus the animals were anesthetized lightly with ether and thyro-parathyroidectomized. After the operation, the animals were on a low iodine diet formulated in accordance with Jolin *et al.*<sup>(12)</sup>, and tap water *ad lib*. The completeness of the operation was judged by inspection of the trachea at necropsy and the absence of appreciable gain in body weight. Animals with unsatisfactory thyroidectomy were not included in the experiment.

At least one month after thyro-parathyroidectomy the animals were sacrificed between 10 AM till 12 noon on the day of proestrus by decapitation. The pituitaries were removed in less than 3 minutes after death, dehydrated in cooled

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acetone and kept in refrigeration. The acetone was changed twice during the first 48 hours. The glands were then air dried, weighted, and kept in a desiccator until the time of bioassay. Five pituitaries of the same group were homogenized together in physiological saline. Appropriate dilutions were then made at the time of bioassay. Rats of the control group were sacrificed at the same age as that of the experimental group, and their pituitaries were treated similarly. Ovarian weights of both experimental and control animals were obtained at the time of autopsy.

In thyroxine ( $T_4$ ) treated group, two weeks after thyro-parathyroidectomy the rats were subjected to a dosage of L- $T_4$  (Sigma Chemical Co.) subcutaneous injection for 2 weeks. The  $T_4$  dosage was in accordance with normal secretion rate ( $2.2 \mu\text{g } T_4/100 \text{ gm body}$ ) which was suggested by previous investigators<sup>(21)</sup>. The completeness of the operation of these rats was examined by 72 hours  $^{131}\text{I}$ -uptake at the time of autopsy. Rats that demonstrated background  $^{131}\text{I}$ -

uptake were then selected. The pituitaries were removed and treated as described, and ovarian weights were also obtained.

**Bioassay:** Pituitary LH concentration was determined by a modification of OAAD method<sup>(17)</sup>. Immature female rats (24–26 days old) of the Sprague-Dawley strain were pretreated with pregnant mare's serum (50 IU, I. P., Gestyl, Organon), and human chorionic gonadotrophin (50 IU, I. P., Pregnyl, Organon) in sequence 72 hours apart to produce fully luteinized ovaries. At the time of assay (7 days after the last injection) the test solutions of either standard LH\* or pituitary extracts were injected into the tail vein. Four hours later these rats were sacrificed and the ovaries were removed. Ascorbic acid concentration of the ovaries was determined by a modification of the method introduced by Mindlin and Butler<sup>(16)</sup>.

Each assay consisted of a four-point analysis which compared two dosages of the pituitary extracts to two dosages of LH standard. A four-fold interval was maintained between the two

TABLE I  
Effect of thyroidectomy on the duration of estrous cycles of female rats†

Ear mark of rats	Cycles and its duration (day) observed after thyroidectomy			
	(1)	(2)	(3)	(4)
42	5	16	5	5
52	5	10	17	
47	4	12	14	
53	4	5	5	14
56	33			
12	4	5	5	9
30	14	6		
27	19			
5	5	4	8	
17	5	18		

Average: 12.03

† At least 4-day regular cycles were observed before thyroidectomy; all rats were sacrificed at the day of proestrus following last estrus observed.

\* The standard LH material, bovine (NIH-LH-B7), was generously supplied by Endocrinological Study Section of National Institute of Health, U. S. A.

dosages of LH (0.4 and 1.6  $\mu\text{g}$ ) and pituitary extracts (1/16 and 1/4 of a gland). Four to five assay animals were used for the LH standard in each dosage level for each assay while pituitary extracts 3 assay animals were used. Pituitary LH concentration was expressed in terms of standard LH, the statistical analysis for each assay being in accordance with the methods by Bliss<sup>(1)</sup> and Burn *et al.*<sup>(4)</sup>.

In the low iodine diet, the Ca supplementary was well supplied<sup>(2)</sup> thus the animals were only with T<sub>4</sub> deficiency.

RESULTS

In the thyro-parathyroidectomized animals the estrous cycles were irregular and in most of the time longer than the 4-day intervals with an average of 12 days (Table 1). However, an estrus could always be expected at the end of the prolonged cycles.

As shown in Table 2, the concentration of pituitary LH in the thyro-parathyroidectomized rats was markedly reduced to about 18% of the normal controls. The ovaries weighted only about half of that of normal controls. There is

TABEL 2  
Effect of thyroidectomy on pituitary LH concentration, pituitary weight and ovarian weight of female rats

Treatment	LH potency ( $\mu\text{g}/\text{mg}$ dry wt pituitary)	$\lambda$ (index of precision)	Weighted mean potency ( $\mu\text{g}/\text{mg}$ dry wt pituitary)	% of control	Pituitary dry weight (mg)	Ovarian weight (mg)
Control	4.59 (3.80-5.60) <sup>a</sup>	0.15	5.57 (2.12-14.70)	—	2.11 $\pm$ 0.06 <sup>b</sup> (15) <sup>c</sup>	63.8 $\pm$ 1.80 (15)
	6.10 (4.73-7.87)	0.14				
Thyroid-ectomy	1.08 (0.56-2.07)	0.14	1.02 (0.64-1.61)	18	1.95 $\pm$ 0.07 (10)	35.8 $\pm$ 2.16 <sup>***</sup> (10)
	0.77 (0.51-1.17)	0.16				

- a. 95% confident limit
- b. Mean $\pm$ S. E.
- c. Number of observations
- \*\*\* Tested by Student's t-test ( $p < 0.001$ )

TABLE 3  
Pituitary LH content and ovarian weight of thyroidectomized ( $\bar{T}$ ) female rats 2 weeks after thyroxine (T<sub>4</sub>) therapy

Treatment	LH content * ( $\mu\text{g}/\text{gland}$ )	% of control	Ovarian weight (mg)
Control	21.4 (13.08-34.98) <sup>a</sup>	—	75.14 $\pm$ 5.81 <sup>b</sup> (5) <sup>d</sup>
$\bar{T}$ +T <sub>4</sub> <sup>c</sup>	15.7 (82.0-30.00)	74	75.51 $\pm$ 6.73 (7)

- \* by combined estimation (Burn *et al.* 1952).
- a. 95% confident limit.
- b. Mean $\pm$ S. E.
- c. 2.2  $\mu\text{g}$  L-T<sub>4</sub>/100gm body weight/day.
- d. Number of observations.

no significant difference between pituitary weights of thyro-parathyroidectomized and normal control rats. The pituitary LH and ovarian weights were restored after two weeks of  $T_4$  treatment (Table 3). Pituitary weights of  $T_4$ -treated group were not recorded.

### DISCUSSION

It is evident from the data presented that thyroidectomy on female rats resulted in: (1) increasing of the duration of estrous cycle (Table 1), (2) reducing of the ovarian weight and (3) reducing of the concentration of pituitary LH (Table 2). As for concentration of pituitary LH, in accordance with NIH-LH-B7, the potency in treated animals showed more than 80% decline. This finding agreed with that of Contopoulos and Koneff<sup>(6)</sup>. In their experiment, the pituitary tissue from thyroidectomized donors was administered to the hypophysectomized recipients, the effectual observation of ICSH content of pituitary was judged by the dose necessary to initiate repairment of interstitial cells of the ovary. In this study, however, the OAAD method was employed, which furnished more direct measurements of the concentration of pituitary LH.

It was demonstrated that on the day of proestrus the LH content of pituitaries obtained between 10-11 AM was not different from that obtained between 4-5 PM on the same day<sup>(24)</sup>. In this experiment, the pituitaries were all obtained within a time interval between 10 AM-12 noon on the day of proestrus, in order to avoid the possible neural cyclic influence *in situ*. The timing for pituitary removal was not put into consideration by previous investigators<sup>(6)</sup>.

In thyroidectomy, or induced hypothyroidism pretreated by thiouracil, the ovarian response to exogenous gonadotrophin was more exaggerated than that of control, however, the response varied with the dosage of gonadotrophin and its duration of treatment. It has been suggested that hypothyroidism leads to an accumulation of gonadotrophin in the blood, and the accumulated gonadotrophin modified the ovarian response to injected gonadotrophin by augmenting the total

effect<sup>(11)</sup>. This does not agree with the data presented with a decreased ovarian weight. However, it is rather difficult to understand that a declined pituitary LH concentration and prolonged estrous cycle, as in this experiment, may be used as indication for accumulation of blood LH. A lack of direct evidence of analysis of blood LH, the conclusion can not be drawn at present. Nevertheless, the reduced ovarian weight in thyroidectomized animals strongly implied the possibility of reduction of LH concentration in blood. The present results agreed with the finding of Gaunt *et al.*<sup>(9)</sup>.

The compensatory augmentation of gonadotrophin in the blood and thyrotrophs transformation in the pituitary have been well documented<sup>(7, 18, 20)</sup>. It is worth noting that both thyrotrophs and gonadotrophs are of basophils origin<sup>(7)</sup>, moreover, in thyroidectomy pituitary thyrotrophs increased while gonadotrophs decreased in number. Evidently, these phenomena are related. Schreiber *et al.*<sup>(23)</sup> indicated that thyroidectomy or induced hypothyroidism inhibited the compensatory of the remaining ovary or adrenal in unilaterally ovariectomized or adrenalectomized rats. They also have suggested that the compensatory secretion of thyroid stimulating hormone (TSH) is the first priority among all tropic hormones. In the  $T_4$ -treated group of this report, the result is in accordance with this explanation, for LH content of the pituitary gland after  $T_4$  treatment was restoring to a great extent. The reason of incomplete restoration may due to the duration of treatment was insufficient (Table 3). With expected estrus appeared at the end of each prolonged cycle in thyroidectomized rat, it is possible that the synthesing and releasing of LH was not completely stopped in pituitary even with deficiency of  $T_4$ . No sufficient data were available to give further explanation on this phenomenon.

It was demonstrated that to maintain normal reproductive function in rats the optimal amount of  $T_4$  present in central nervous system (CNS) is crucial<sup>(10)</sup>. This was expressed in terms of initiation of vaginal cornification and ovulation.

This finding agreed with the proposal of Gaunt *et al.*<sup>(9)</sup> that reduction of thyroid function blocked the hypothalamohypophyseal-gonad axis but the exact locus remained to be investigated.

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## 甲狀腺切除對大白鼠腦下垂體排卵素濃度之影響

萬家茂 黃基礎

充份鈣質的補充下大白鼠於甲狀腺及副甲狀腺切除後，其腦下垂體中排卵素的濃度有明顯減低的現象，且性週期時間延長，卵巢重量亦減輕，此等現象皆可以甲狀腺素之補給而恢復，與此有關之機制于文中加以討論。