CHRONIC EFFECTS OF TRIIODOTHYRONINE
ON THYROTROPHIN LEVELS IN THYROIDECTOMIZED RATS

By
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ABSTRACT

In order to study the mechanism of the paradoxical potentiating effect of small doses of thyroid hormone on propylthiouracil goiter growth in rats, serum and pituitary thyrotrophin (TSH) titers were measured after small and larger doses of triiodothyronine ($T_3$) in thyroidectomized rats. A small dose of $T_3$, 4 $\mu$g daily, sufficient to maintain normal oxygen consumption, caused elevated pituitary and serum TSH titers when compared to controls. Reduced titers of TSH resulted from a larger dose of $T_3$, 16 $\mu$g daily. It is concluded that the goitrogenic action of small doses of thyroid hormone is not limited to an interaction peculiar to propylthiouracil administration but may be the result of direct stimulation of increased synthesis and secretion of TSH by the pituitary gland and is seen even in the absence of thyroid tissue or propylthiouracil. It is hypothesized that small amounts of thyroid hormone are necessary for optimal or maximal synthesis and secretion of TSH. These effects may be mediated directly at the pituitary gland or at a suprapituitary level. It was also found that stalk-median eminence TSH titers were significantly increased in the thyroidectomized rats that received a large dose of $T_3$, as compared to those that received no $T_3$, i.e. the effects were the opposite of those seen in the pituitary gland.

Small doses of thyroid hormones exert a biphasic effect on the formation of goiters; very small amounts potentiate the goitrogenic effect of propylthiouracil
(PTU), while somewhat larger doses produce the expected antigoitrogenic effect (Sellers et al. 1953; Sellers & Schönbaum 1962, 1965).

The stimulation of goiters by small doses of thyroid might be a peculiar interaction with PTU, or it might be a direct thyroidal or nutritional effect or it might be mediated by enhancing the secretion of thyrotrophin (TSH). In order to study this latter possibility we investigated the effects of L-triiodothyronine (T₃) on TSH formation and release in thyroidectomized rats. Thyroidectomized rats were chosen in order to avoid the use of PTU which is known to introduce other complex variables in the rat, including both impaired absorption of oral thyroxin, and depression of the metabolic effectiveness of thyroxine (Escobar del Rey & Morreale de Escobar 1961; Morreale de Escobar & Escobar del Rey 1967).

We found that T₃ caused elevated hypophyseal and serum TSH titers if given in small doses, but that a larger dose of T₃ strongly reduced both of these titers.

**MATERIALS AND METHODS**

Male Sprague-Dawley rats raised in the colony of the Pacific Northwest Research Foundation were thyroidectomized when about one month old. Radioiodine (¹³¹I) was used to accomplish thyroidectomy since surgical thyroidectomy is usually defeated by eventual hypertrophy of residual tissue in long term experiments. After a week on a low iodine diet, each rat received 300 microcuries of carrier-free ¹³¹I sodium iodide intraperitoneally. Six weeks later, when they weighed between 180 and 220 grams, the thyroidectomized rats were divided into three groups which were given a daily dose of approximately 0.4 or 16 micrograms of T₃ (Nutritional Biochemicals Corp.) dissolved in alkaline isotonic saline and added in the drinking water. Oral administration was deemed necessary to provide a constant intake over the full 24-hour period and to avoid the stress that would occur with daily injections over the prolonged period of this experiment. The volume of water was adjusted so that the water bottles were completely emptied in 24 hours with only sporadic exceptions. The effectiveness of this method of oral administration was demonstrated in a preliminary experiment (see below) and has since been shown to be effective by others (Bauman & Turner 1966).

An additional control group of 12 rats was not thyroidectomized and was given plain water and Purina Lab Chow throughout the experiment.

In a preliminary experiment, lasting three weeks, a dose of T₃ was established that would suppress the goitrogenic effect of 0.025% PTU in the drinking water. It was found that 2 micrograms of T₃ administered daily in addition to the PTU in drinking water would keep the thyroid weight at 6.8 ± 0.8 mg* while PTU alone, caused goiters with a mean weight of 59.7 ± 3.2 mg*. During this period PTU did not affect body growth; the average body weight in the PTU group increased from 63 to 157 g while the PTU group, receiving T₃, increased from 67 to 149 g. The

* Mean ± standard error.
higher doses used in the long-term study were then calculated according to the greater weight of adult rats.

Oxygen uptakes were measured after 76 weeks of treatment, using the method of Levey & Roberts (1962). After 90 weeks, the rats were anaesthetized with pentobarbital, 3 mg/100 g body weight, exsanguinated via the abdominal aorta, and the pituitary gland, thyroids, adrenals, kidneys and heart removed and weighed. Tracheae were inspected and confirmed the absence of thyroid tissue in every instance. Sera, pituitary glands and the stalk-median eminence area from each group were pooled and stored, frozen, for subsequent TSH assay. TSH was assayed by the method of Bakke et al. (1957). Samples were assayed at least three times and the independent assays combined according to the method of Bliss (1952).

RESULTS

The effects of T₃ treatment on the organ weights and oxygen uptake of radiothyroidectomized rats are shown in Table 1.

Body Weight
None of the thyroidectomized rats receiving T₃ reached the body weight of the intact controls.

Oxygen Consumption
The thyroidectomized rats receiving the high dose of T₃ had the same rate

<table>
<thead>
<tr>
<th>Description</th>
<th>Intact controls No treatment</th>
<th>Thyroidectomized rats No T₃</th>
<th>4 µg T₃/d</th>
<th>16 µg T₃/d</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial/Final number of rats</td>
<td>14/10</td>
<td>10/5</td>
<td>10/7</td>
<td>10/6</td>
</tr>
<tr>
<td>Range of life span (days)</td>
<td>27–655</td>
<td>90–655</td>
<td>31–655</td>
<td>312–655</td>
</tr>
<tr>
<td>Initial body weight (g)</td>
<td>37 ± 0.3</td>
<td>38 ± 2</td>
<td>38 ± 7</td>
<td>41 ± 1</td>
</tr>
<tr>
<td>Final body weight (g)</td>
<td>502 ± 11</td>
<td>395 ± 21</td>
<td>426 ± 12</td>
<td>492 ± 9</td>
</tr>
<tr>
<td>Thyroid weight (mg)</td>
<td>24.5 ± 1.3</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Pituitary weight (mg)</td>
<td>14.3 ± 0.93</td>
<td>21.3 ± 2.00</td>
<td>25.6 ± 2.31*</td>
<td>13.7 ± 0.41</td>
</tr>
<tr>
<td>Adrenal weight (mg)</td>
<td>51.5 ± 4.0</td>
<td>39.2 ± 1.11</td>
<td>37.7 ± 0.70</td>
<td>54.8 ± 0.84</td>
</tr>
<tr>
<td>Kidney weight (g)</td>
<td>3.59 ± 0.22</td>
<td>1.95 ± 0.13</td>
<td>2.30 ± 0.14</td>
<td>4.39 ± 0.53</td>
</tr>
<tr>
<td>Heart weight (g)</td>
<td>1.62 ± 0.05</td>
<td>0.88 ± 0.05</td>
<td>0.89 ± 0.05</td>
<td>1.55 ± 0.04</td>
</tr>
<tr>
<td>Oxygen consumption (ml O₂/h/100 cm²)</td>
<td>100 ± 9</td>
<td>60 ± 5</td>
<td>77 ± 4</td>
<td>114 ± 7</td>
</tr>
</tbody>
</table>

* Excluding one pituitary tumour weighing 180 mg.

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of oxygen consumption as the intact controls, while those on the low dose consumed significantly more oxygen than the rats without any T₃ supplement.

**Organ Weights (Except Pituitary)**

Thyroidectomized rats on the high dose of T₃ had organ weights similar to those of the intact controls, while the low dose of T₃ was essentially without effect on organ weights.

**Pituitary Weights**

Thyroidectomy caused a significant increase of the pituitary weight. Supplementation with a low dose of T₃ caused a slight further increase of the average pituitary weight, while the high dose caused a return of the pituitary weight to the level of the untreated control rats.

**TSH Estimates**

The TSH estimates are summarized in Table 2. Thyroidectomy alone caused a more than six-fold increase of the pituitary TSH content and a more than forty-fold increase of the serum TSH level. Administering a small amount of T₃ further increased pituitary and serum TSH by more than 50%. The larger dose of T₃ caused suppression of the pituitary TSH to far below control levels and serum TSH to a concentration that was, just as the untreated controls, below the sensitivity of the assay method. The stalk-median eminence TSH content was significantly increased in the thyroidectomized rats that received the large dose of T₃, as compared with those that received no T₃.

| Table 2. Effects of Triiodothyronine on the Thyrotrophin Levels of Thyroidectomized Rats. |
|---------------------------------|-----------------------------|-----------------------------|-----------------------------|-----------------------------|
| Final number of rats | Intact controls No treatment | Thyroidectomized rats No T₃ | 4 µg T₃ | 16 µg T₃ |
| 10 | 6* | 6 |
| Mean pituitary weight (mg) | 14.3 ± 0.93 | 21.3 ± 2.00 | 25.6 ± 2.31 | 13.7–0.41 |
| Pituitary TSH content (mU) | 288 | 1842 | 3132 | 7.95 |
| Serum TSH (mU/ml) | < 0.1 | 3.98 | 7.08 | < 0.1 |
| 95% Confidence limits | 3.1–5.0 | 4.98–10.1 |
| Stalk-median eminence (mU) | 1.07 | 0.73 | 1.18 | 1.48 |
| 95% Confidence limits | 0.81–1.43 | 0.53–1.00 | 0.93–1.50 | 1.16–1.87 |

* The rat bearing a pituitary tumour (180 mg) has been excluded.
The intermediate group had an intermediate titer, but the 95\% confidence limits overlapped the adjacent groups.

**DISCUSSION**

*Introduction*

The »goitrogenic« effect of thyroid hormones has been under study by Sellers and associates for more than ten years. These workers have shown under a variety of experimental conditions, but always in chronic studies involving the feeding of thyroid hormones, that small doses of thyroid preparations stimulate drug-induced goiter formation. *Sellers & Schönbaum* (1965) suggested that the action of thyroxine in potentiating goitrogenesis was likely related to an effect produced on the hypophysis or higher centers, rather than an effect on the thyroid or peripheral tissues. The experiment described in this paper demonstrates that small amounts of thyroid hormone, in this instance T₃, administered chronically, stimulates thyrotrophic activity in thyroid-ectomized animals.

*Methodology*

In this experiment, administration of T₃ in drinking water rather than by injection or addition to the diet was chosen. The risks, stress and inconvenience of multiple injections over a period of many months were considered a greater hazard than the possible loss of activity or accuracy due to irregular oral intake. Day-to-day variations of the intake would be equalized by the long duration of treatment. In a preliminary experiment, described in the section on Methods, the adequacy of the chosen dose of T₃ was ascertained.

The bio-assay for TSH used in this study has proven reliable in many previous studies (*Bakke et al.* 1962; *Bakke & Lawrence* 1962, 1964). Moreover, it has recently been demonstrated that an antiserum to human TSH* and one to bovine TSH** will equally neutralize the assay response to rat TSH of either pituitary or stalk-median eminence origin. In addition, both pituitary and stalk-median eminence extracts give parallel assay responses and both were recovered to the same extent, 93\% (*Bakke*, unpublished) by our salt and acid extraction method (*Bakke et al.* 1961). The stalk-median eminence extract gave TSH responses not only when assayed by the method of *Bakke & Lawrence* (1964) but also when tested by the method of Kirkham as modified by *Desbarats-Schönbaum et al.* (1967). In the latter, the response was also blocked by an antiserum to bovine TSH (*Bakke & Desbarats-Schönbaum*, unpublished).

* Prepared and generously provided by Dr. W. D. Odell.
** Prepared and generously provided by Dr. F. Greenspan.
It would appear that the stalk-mediated eminence thyroid stimulating material is very similar, if not identical, with pituitary TSH.

**Effects of T3 in Thyroidectomized Rats**

The small dose of T3 caused slight increases of the average total body weight and of the pituitary and kidney weights. The large dose, however, markedly reduced the pituitary weight down to that found in intact, untreated rats. This dose also brought adrenal, heart and kidney weights up to normal levels. This dose of T3 did not increase the total body weight of the group compared to that of the untreated controls. It is believed that this is a result of the fact that food restriction in the young rat, even for a brief time, produces life-long subnormal growth, even though unlimited food is offered subsequently (*Kennedy* 1957). All of the radiothyroidectomized rats had been subjected to a nutritionally inadequate low iodine diet for one week prior to their dose of radioiodine. The adequacy of the large dose of T3 as a replacement dose is evidenced by the normal values for oxygen uptake, the suppression of serum TSH and reduced pituitary weight. Similarly, the inadequacy of the »small dose« of T3 as total replacement, but as an effective intermediate dose is evidenced by oxygen uptake, body and kidney weights.

**Effects of T3 on TSH Titers**

The data of Table 2 clearly show that thyroidectomy caused large increases of pituitary weight, of TSH content and of serum TSH. A small amount of T3 further increased both TSH content of the pituitary and serum TSH levels. A larger dose of T3 suppressed pituitary TSH contents to far below that of intact control rats, while serum TSH in both these groups was below detectable levels. The TSH content of the stalk-mediated eminence tissue varied only slightly in spite of more than 300-fold changes in hypophyseal TSH. This suggests that the stalk-mediated eminence TSH is independent of change in pituitary TSH and certainly is not the reflection of passive mechanical leakage during the removal of the tissues. The factors controlling stalk-mediated TSH levels are unknown and are under further study. *Bakke & Lawrence* (1964) and *Van Rees* (1966) studied short term effects of thyroxine administration in PTU-treated or thyroidectomized rats. These workers observed effects of low doses of thyroxine somewhat similar to those reported here. However, as long as the mechanism of the TSH secretion stimulating effect of small amounts of thyroid hormones is not fully understood, it is preferable not to attempt to equate results from short and long term studies.

As noted in Table 1, a single animal which received 4 micrograms of T3 per day revealed a pituitary tumour at autopsy. This tumour weighed 180 mg and contained a total of 21 770 mU (18 680 - 25 390) of TSH, or 121 mU/mg wet weight. The remaining members of this group had an average concentra-
tion of 122 mU/mg. This remarkable identity in TSH titers of the presumably physiological and of the neoplastic tissues, is of interest.

REFERENCES

Levey H. A. & Roberts S.: Endocrinology 71 (1962) 244.

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