RELATIONSHIP BETWEEN PEROXIDASE ACTIVITY AND SERUM TSH, T₄ AND T₃ LEVELS IN RATS IN THE COURSE OF IODINE DEFICIENCY

By

B. M. Nataf, P. Fragu and S. Ben Othman

ABSTRACT

The relationship between peroxidase activity and serum TSH, T₄ and T₃ levels was investigated in the course of iodine deficiency in rats. Rats were maintained either on a control diet with a relatively high iodine content (600 μg/kg of ¹²⁷I), or on a low iodine diet (30 μg/kg of ¹²⁷I). Twenty days after the low iodine treatment, the thyroid iodine [¹²⁷I] concentration was half that of control value (647 ± 52 and 1241 ± 72 μg/g of wet weight, respectively). However, no significant changes in serum T₄, T₃, TSH were found at 20 days even though an early increase in peroxidase activity was observed. It was only at 35 days of iodine deficiency, when the concentration of iodine in the gland averaged 260 μg/g of wet weight that serum T₄ and TSH levels started to be significantly modified. From day 35 to day 70, a significant and progressive decrease of plasma T₄ concentration was observed, and it levelled off thereafter. The changes of serum T₃ were much smaller than those of T₄. A significant increase in serum TSH level was noted at 35 days. Thereafter TSH levels increased rapidly and progressively (205 % increase over control at 70 days and 643 % at 80 days). From day 35 until day 80 of the low iodine treatment, the thyroid peroxidase activity and the serum TSH level varied concomitantly.

Our results suggest that for an iodine content between 5 and 2 μg per thyroid gland, the high cellular peroxidase activity observed could be correlated with an increase in circulating TSH, due to a decrease of T₄. In contrast, in the early period of iodine deficiency, no correlation was found between peroxidase activity and serum T₄, T₃ and TSH levels.

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The evolution of peroxidase activity in the course of iodine deficiency in rats has previously been described and a high cellular activity has been found (Fragu & Nataf 1976). It has then been suggested that the specific enzyme induction observed during the iodine deficiency in rats is comparable to that found in human sporadic goitre (Fragu & Nataf 1977).

In rats maintained on a normal diet to which 6-propyl-2-thiouracil (PTU) has been added, changes in peroxidase activity have been reported and it has been suggested that the increase in enzyme activity may be correlated to increased blood TSH level (Nagataki et al. 1973; Yamamoto & De Groot 1974). The aim of the present investigation was to verify this hypothesis.

It has been shown that various commercial low iodine diets differ largely in their ability to induce goitre in rats and to affect various parameters of thyroid function and that these differences could be correlated with differences in the iodine content of the diets (Riesco et al. 1976). Therefore, it was interesting to follow changes in serum TSH, T₄ and T₃ concentrations in rats maintained on a low iodine diet and to relate these parameters to the modifications observed by Fragu & Nataf (1976) in peroxidase activity.

**Materials and Methods**

Female adult Wistar rats (average weight: 190 g) were maintained either on a control diet (U.A.R. 113) with a relatively high iodine content (600 μg/kg of ¹²⁷I) or on a low iodine diet (LID), containing 30 μg/kg of ¹²⁷I (Triantaphyllidis diet Anvar License 69 195 00). The animals were killed by cardiac puncture on days 10, 20, 35, 70, 80 and 120 after the beginning of treatment. The thyroid glands of each group were excised, weighed and pooled. Their peroxidase activity was measured, using a peroxidegenerating system, by oxidation of iodide and by iodination of bovine serum albumin (Fragu & Nataf 1976). The concentration of stable iodine [¹²⁷I] in the thyroid tissue was measured by the method described by Comoy (1967), using a technicon autoanalyzer.

The blood of each animal was collected, centrifuged, frozen and stored for later TSH, T₄ and T₃ determinations. Serum TSH level was determined by a double antibody radioimmunoassay technique with NIAMDD rat TSH 1–2 and TSH 1–3 for iodination as well as for standards (the kit was obtained from NIAMDD rat pituitary hormone programme). Total T₄ and T₃ were determined by radioimmunoassay, using commercial kits (Lepetit Laboratory).

**Results**

The iodine content of the gland decreased rapidly and progressively during the first 35 days of iodine deficiency, and continued to fall at a slower rate thereafter (Fig. 1). On day 20, the iodine content was about half that of the control value (647 ± 52 and 1241 ± 72 μg/g of wet weight, respectively), while, between 35 and 70 days, the ¹²⁷I contents of the gland were respectively 259 ± 42.
and 147 ± 26 µg/g. Serum TSH levels were not different from those of controls on days 10 and 20 of the low iodine treatment (Table 1 and Fig. 2) when an early increase in peroxidase activity was observed (Fragu & Nataf 1976). From day 20, serum TSH increased and by day 35 the concentration of TSH was significantly higher than that of control (71 ± 7 % increase over control). The serum TSH level continued to increase at a steady rate until day 70 (205 ± 15 % increase over control); and thereafter, at a very fast rate attaining 643 ± 33 % on day 80. Between days 35 and 80, serum TSH levels and iodide-peroxidase activity evolved parallely (Fig. 2).

As for TSH, no significant changes in plasma T₄ and T₃ were observed before day 20 of the treatment (Table 1). From day 35 to day 70, a significant and progressive decrease of serum T₄ concentration was found. Then the level of T₄ remained steady between days 70 and 80. The modifications of T₄

![Fig. 1.](image)

Changes in thyroid iodine concentration (mean ± SEM) during the low iodine diet treatment. Number of determinations: control: 22; 10 and 20 days of LID: 8; 35 days of LID: 19; 70 days of LID: 14; 80 days of LID: 19; 120 days of LID: 20.
Table 1.
Serum TSH, T₄ and T₃ levels in the course of iodine deficiency.

<table>
<thead>
<tr>
<th></th>
<th>TSH ng/ml</th>
<th>T₄ µg/100 ml</th>
<th>T₃ ng/100 ml</th>
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<tbody>
<tr>
<td>Control</td>
<td>11.4 ± 0.7 (80)</td>
<td>5.8 ± 0.3 (31)</td>
<td>133 ± 7 (14)</td>
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<tr>
<td>Rats fed on a low iodine diet for:</td>
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<tr>
<td>10 days</td>
<td>9.6 ± 0.8 (41)</td>
<td>5.0 ± 0.3 (20)</td>
<td>130 ± 11 (15)</td>
</tr>
<tr>
<td>20 days</td>
<td>11.4 ± 1.2 (28)</td>
<td>5.2 ± 0.3 (15)</td>
<td>149 ± 17 (10)</td>
</tr>
<tr>
<td>35 days</td>
<td>19.5 ± 1.8* (26)</td>
<td>3.8 ± 0.3* (19)</td>
<td>168 ± 8 (12)</td>
</tr>
<tr>
<td>70 days</td>
<td>35.0 ± 3.8* (25)</td>
<td>1.8 ± 0.2* (13)</td>
<td>157 ± 7 (7)</td>
</tr>
<tr>
<td>80 days</td>
<td>85.0 ± 9.6* (35)</td>
<td>2.1 ± 0.1* (18)</td>
<td>130 ± 10 (7)</td>
</tr>
<tr>
<td>120 days</td>
<td>194 ± 20.4* (34)</td>
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Mean values of TSH, T₄ and T₃ determination ± standard error of the mean. The number of determinations is indicated in brackets.

* Significantly different from control (P < 0.001).

Fig. 2.
Per cent increase or decrease in serum TSH, T₄ and T₃ levels and in thyroid iodide-peroxidase activity in the course of iodine deficiency.
(35 ± 4 % decrease from control at 35 days and 69 ± 5 % at 70 days) were smaller than those of TSH (Fig. 2). There was a highly significant \( P < 0.001 \) negative correlation of serum \( T_4 \) with serum TSH with a correlation coefficient of 0.49, in agreement with Fukuda et al. (1975).

No significant changes of serum \( T_3 \) were found in the course of iodine deficiency (Fig. 2). At 35 days a small increase in \( T_3 \) was observed \((22 ± 5 \% \) increase over control\), and the serum \( T_3 \) concentration returned slowly to normal values at 80 days (Table 1).

**DISCUSSION**

Thyroid investigators commonly observe large variations in thyroidal responses to commercially available low iodine diets and it seemed reasonable to attribute these variations to differences in the iodine content of the diets (Riesco et al. 1976). Our control diet contained a relatively high level of iodine and our low iodine diet contained 30 \( \mu g/kg \) of \( ^{127}I \) which is higher than that used by other investigators (Riesco et al. 1977).

Under our experimental conditions the thyroid \( ^{127}I \) concentration decreased quite rapidly after the low iodine treatment, as previously shown (Studer & Greer 1965; Berthier & Lemarchand-Béraud 1976); however, the rate of fall of thyroid \( ^{127}I \) was more rapid under their experimental conditions. Recent studies by Riesco et al. (1977) showed even a more rapid decrease in thyroid \( ^{127}I \) content using a diet which contained only 10–20 \( \mu g \) of iodine per kg of diet; it is noteworthy that the iodine thyroid content of their control rats was only half that of ours.

In our hands, no significant changes in serum \( T_4 \), \( T_3 \) and TSH were found after 20 days of low iodine treatment. It was only after 35 days of iodine deficiency, when the concentration of iodine in the gland averaged 260 \( \mu g/g \) that serum \( T_4 \), \( T_3 \) and TSH levels started to be modified. Serum \( T_4 \) levels decreased quite rapidly until day 70, but an earlier and/or more rapid decline has been found by others, as early as the first week (Riesco et al. 1977; Berthier & Lemarchand-Béraud 1976) or during the second week of LID (Fukuda et al. 1975). However, after approximately 1 month of LID, the serum \( T_4 \) level of our animals was in the same range as that of Berthier & Lemarchand-Béraud (1976) who used a similar low iodine diet. At 80 days, the serum \( T_4 \) level was about 2 \( \mu g/100 \) ml while for others it was undetectable at the end of 1 month (Abrams & Larsen 1973; Fukuda et al. 1975; Riesco et al. 1977). These discrepancies can be attributed, at least in part, to the various iodine contents of control and LID diets used by these investigators, as well as to the thyroid iodine content of their control rats, and to its rate of change in the course of iodine deficiency.

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The changes of T\(_3\) are smaller than those of T\(_4\); in our experiments, a slight increase occurred at 35 days of iodine deficiency. Berthier & Lemarchand-Béraud (1976) described a small but rapid increase in plasma T\(_3\), reaching a maximum value on the 14th day, whereas Volpert & Werner (1972) reported a significant decline in serum T\(_3\) in rats that had been on LID for 4-5 weeks. However, other investigators found that the T\(_3\) level remained at a nearly constant level throughout the first month of iodine deficiency (Fukuda et al. 1975; Riesco et al. 1977) and even after 2 and 3 months of restriction of iodine intake (Abrams & Larsen 1973). Therefore it seems that in iodine deficiency serum T\(_3\) can be more easily maintained in the normal range by an adaptative mechanism than can T\(_4\), as was already suggested by Fukuda et al. (1975).

In agreement with previous reports by Studer & Greer (1965) using the MacKenzie assay, we observed no early modification of serum TSH level. More recently, using different control and/or LID diets, Fukuda et al. (1975) and Riesco et al. (1977) found that blood TSH displayed a marked increase after 15 days of iodine deficiency. In our hands, the increase of plasma TSH concentration became significant only at 35 days. At day 35 until day 70 after the beginning of the LID treatment, the thyroid peroxidase activity, the thyroid weight (Fragu & Nataf 1976) and the serum TSH level varied concomitantly. However, at day 70 and until day 80, the increase of thyroid peroxidase activity (321 ± 48 and 689 ± 135%/over control, respectively) was quite identical to that of TSH level (205 ± 15%/over control on day 70 and 643 ± 33%/over control on day 80) while the extent of increase in thyroid weight was smaller (157 ± 20%/over control on day 70 and 230 ± 26%/over control on day 80) (Fragu & Nataf 1976). Our results suggest that for an iodine content between 5 and 2 μg/thyroid, the high cellular peroxidase activity, could be partially correlated with an increase in circulating TSH, due to a decrease of T\(_4\).

In contrast, during the early period of iodine deficiency, no correlation existed between thyroid peroxidase activity and serum TSH level, since already on day 10, the enzymatic activity had increased (Fragu & Nataf 1976) whereas TSH levels showed no significant change. This agrees with Berthier & Lemarchand-Béraud (1976) who found no increase in serum TSH level after 1 month of iodine deficiency.

It is conceivable that the early increase in peroxidase activity could be related to some slight modifications in the adenylyclase-cyclic AMP system during the first days of iodine deficiency. Berthier & Lemarchand-Béraud (1976) noted, in fact, an early (4th day) but transitory increase in cAMP which returned rapidly to normal values. However, Zakarija et al. (1973) did not find any increase in cAMP at day 17 of the low iodine treatment. Since these results seem contradictory, more information is needed.

Some years ago Bray (1968) indicated that iodine depletion increases the sensitivity of the thyroid gland to the effects of TSH. These results were con-
firmed more recently by Rapoport et al. (1975, 1976) who indicated that the iodine content of the gland seems to regulate the response of adenylylclase-cyclic AMP systems to TSH stimulation.

The increased sensitivity of the iodine-depleted thyroid gland may provide a possible explanation for the thyroid enlargement and for the increase in cellular peroxidase activity during the early period of iodine deficiency without requiring an increase in the level of circulating TSH. This could also explain the increase in peroxidase activity observed in sporadic goitre (Fragu & Nataf 1977), when the serum TSH level of the patients were in the normal range.

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REFERENCES


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