Effects of Thyroidectomy and Administration of Propylthiouracil (PTU) or Thyrotropin (TSH) to Pregnant Rats on the Functional Development of the Fetal Thyroid Gland
An Immunohistochemical Study

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Abstract

In order to elucidate the maternal factors influencing the functional development of the fetal rat thyroid gland, pregnant rats were subjected to either thyroidectomy or administration of PTU or TSH and the thyroid glands of the fetuses were examined chronologically by immunohistochemistry to detect thyroglobulin (Tg), T₄ and T₃.

In the group undergoing thyroidectomy, the occurrence of immunoreactive Tg, T₄ and T₃ was the same as in the control group in spite of slight retardation of the development of the thyroid gland. On the other hand, PTU administration caused remarkable degeneration of the hyperplastic epithelium of the follicles, where immunoreactivity of T₄ and T₃ was barely detectable, suggesting a transplacental effect of PTU on the fetal thyroid gland. However, Tg remained unaffected and was stained as well as in the controls.

Injection of TSH led to a delay in the occurrence of T₄ and T₃ by one day, probably due to increased levels of thyroid hormone from the stimulated thyroid gland of the mother rats.

It is very important to elucidate the point at which the mammalian thyroid gland begins to synthesize hormones during fetal life, as well as the mechanism of its control. In addition to electron microscopic studies and biochemical analysis of the iodine uptake and in vitro synthesis of iodides, we applied immunohistochemical detection of thyroglobulin (Tg) and thyroid hormone to the fetal rat thyroid gland. It was found that immunoreactive Tg first appeared in the cytoplasm of the immature thyroid epithelium on the 15th day of gestation, followed by the appearance of T₄ and T₃ in the lumen of primitive follicles 2 days later (Kawaoi and Tsuneda, 1985).

This paper describes the effects of thyroidectomy and administration of PTU or TSH to pregnant rats on the functional development of the fetal thyroid gland based on results for the immunohistochemistry of Tg and thyroid hormone.

Materials and Methods

Pregnant rats of Wistar strain were used. The day following overnight mating was regarded as the 1st day of gestation, and the thyroid
glands of fetuses from the 14th to 21st day of gestation were examined by immunohistochemical methods.

The following experimental groups were prepared, each consisting of 10 to 15 animals.

**Group 1.** Untreated controls.

**Group 2.** The animals were thyroidectomized on the 8th day of gestation.

**Group 3.** Five mg of propylthiouracil, PTU (Thiouracil tablets, Tanabe Pharmaceutical Co., Tokyo, Japan), in 0.5 ml of water was administered daily via a stomach tube from the 8th day of gestation.

**Group 4.** The animals were injected with 500 mIU of thyroid stimulating hormone, TSH (thyrotrophic hormone from the bovine pituitary, Sigma Chemical Co., St. Louis, M. O., U.S.A.).

In each experiment, fetuses were taken out chronologically. The thyroid glands were removed with other neck organs, fixed immediately in Zamboni's solution overnight at 4°C, and embedded in paraffin as usual. Serial or semi-serial section 3-4 μm thick were cut and stained with haematoxylin and eosin, and subjected to the immunoperoxidase technique for Tg, T4 and T3.

An indirect immunoperoxidase method was employed using anti-T4 and anti-T3 rabbit antisera provided for radio-immunoassay at 500 tubes per ml (Cappel Laboratories, Cochranville, PA., and E. Y. Laboratories, Inc., San Mateo, CA., U.S.A.), anti-rat thyroglobulin rabbit antisera which were produced in our laboratory following the procedure of Chopra et al. (1971) and were absorbed of cross-reactivity with T4 and T3 by affinity column chromatography (Pensky and Marshall, 1969), and horseradish peroxidase labelled antirabbit IgG goat immunoglobulin prepared in our laboratory according to the method of Nakane and Kawaoi (1974). The deparaffinized tissue sections were incubated with the antisera and labelled antibody as described previously (Kawaoi and Tsuneda, 1985), and visualized by incubation in Graham and Karnovsky's solution (Graham and Karnovsky, 1966), followed by counter staining with hematoxylin. Negative controls for the immunostain were also prepared, and confirmation of the absence of endogenous thyroid peroxidase activity (Strum and Karnovsky, 1970; Tice and Wollman, 1974) was performed as described previously (Kawaoi et al., 1983).

**Results**

**Group 1.** Untreated controls.

Tg was sporadically immunostained for the first time on the 15th to 16th day of gestation in the cytoplasm of the immature epithelium of the thyroid glands which had no follicular lumen yet clearly identifiable at the light microscopic level. The amount of Tg positive epithelium was increased later on, and immunostainability was also intensified.

T4 and T3 appeared on the 17th day filling the tiny lumen of thyroid follicles with positive Tg.

The follicles continued to develop towards the end of gestation increasing the size of the lumen where, in contrast to Tg which was located in both the lumen and the cytoplasm of the epithelium, most of immunoreactive T4 and T3 was confined.

These findings confirmed the results reported by us previously (Kawaoi and Tsuneda, 1985).

**Group 2.** Thyroidectomy.

Maturation of the thyroid glands of fetuses from thyroidectomized mothers, in association with systemic retardation of somatic development, was significantly delayed by the prolonged date of opening of the follicular spaces and immaturity of the epithelium.

However, immunoreactivity of Tg, T4 and T3 occurred regularly as in the controls. That is, Tg was observed on the 15th day and both T4 and T3 on the 17th day of gestation, with decreased frequency of positive follicles and weak stainability of the latter (Figs. 1a and 1b.) The staining pattern on the 18th day in this group corresponded approximately to that on the 17th day in the controls (Figs. 2a and 2b), although the stainability increased gradually after the 19th day.
Figs. 1a and 1b. Serial sections of foetal thyroid of the group 2 (thyroidectomy) at the 17th day of gestation, stained for Tg (1a) and T₄ (1b). The majority of follicular epithelium is positive for Tg, and only two primitive follicles show positive for T₄ (arrows). The nucleus of the epithelium is lightly stained with hematoxylin. ×600.

Group 3. PTU administration.

Tg appeared positive on the 15th day as in the controls with normal development of the thyroid gland until the end of the 17th day of gestation (Fig. 3).

After the 18th day, hyperplasia with an unclear follicular structure and degenerative changes in the epithelium were observed and increased in severity day by day. Although Tg was clearly demonstrated even in the degenerative epithelium, both T₄ and T₃ were completely negative on the 17th day (Figs. 4a and 4b). T₃ was exceptionally positive in a few fetuses on the 18th day, but remained negative in most of the animals until birth, also with negative T₃ (Figs. 5a and 5b).

Figs. 2a and 2b. Serial sections of foetal thyroid of the group 2 at the 18th day of gestation, stained for Tg (2a) and T₄ (2b). The number of T₄ positive follicles is increased (arrows). ×600.

Fig. 3

Fig. 3. Foetal thyroid of group 3 (PTU administration), at the 16th day of gestation, stained for Tg. ×600.
Group 4. TSH administration.

Tg appeared on the 15th day of gestation, and then increased in stainability together with the amount of positive epithelium (Fig. 6). The occurrence of T₄ and T₃ was delayed for one day (Figs. 7a and 7b), and a few follicles in 18-day-old fetuses exhibited positive staining in the lumen (Figs. 8a and 8b).

The thyroid gland developed normally later on, and the staining patterns of Tg, T₄ and T₃ revealed no significant difference from those of untreated controls.

The time course of the occurrence of Tg, T₄ and T₃ in the fetal thyroid glands in each experimental group is summarized in Table 1.
Figs. 7a and 7b. Serial sections of foetal thyroid of group 4 at the 17th day of gestation, stained for Tg (7a) and T₄ (7b). No positive T₄ staining is observed at this period. ×600.

Figs. 8a and 8b. Serial sections of foetal thyroid of group 4 at the 18th day of gestation, stained for Tg (8a) and T₄ (8b). Some follicles positive for T₄ appear from this period (arrows). ×600.

### Table 1. Occurrence of immunostainable T₃, T₄ and Tg in foetal rat thyroid glands of each experimental group

<table>
<thead>
<tr>
<th>Day of gestation</th>
<th>15</th>
<th>16</th>
<th>17</th>
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<th>19</th>
<th>20</th>
<th>21</th>
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<tbody>
<tr>
<td>Group 1 (control)</td>
<td>T₃ :</td>
<td>-</td>
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<td>±</td>
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<tr>
<td></td>
<td>T₄ :</td>
<td>-</td>
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<td>+</td>
<td>+</td>
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<tr>
<td></td>
<td>Tg :</td>
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<td>+</td>
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<td>Group 2 (thyroidectomy)</td>
<td>T₃ :</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>±</td>
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<td></td>
<td>T₄ :</td>
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<td></td>
<td>Tg :</td>
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<td>Group 3 (PTU administration)</td>
<td>T₄ :</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>(±)</td>
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<td>Tg :</td>
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<tr>
<td>Group 4 (TSH administration)</td>
<td>T₄ :</td>
<td>-</td>
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<td>-</td>
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<td></td>
<td>Tg :</td>
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± : positive, ± : weakly or partially positive, - : negative, (): exceptional
Discussion

It is important to elucidate in detail the functional development process of the thyroid gland during fetal life, since the thyroid hormone is known to stimulate the development of the nervous and skeletal systems (Eayrs, 1971). For this purpose, various approaches have been adopted such as electron microscopy (Ishikawa, 1965), autoradiographic studies of the uptake and organification of iodine (Carpenter, 1959; Rémy et al., 1980), in vitro analysis of the uptake of iodine and synthesis of hormone (Nataf et al., 1965; Nataf, 1968), immunohistochemical demonstration of Tg in the fetal thyroid gland (Feldman et al., 1961), etc. The present authors employed the immunoperoxidase technique and demonstrated that immunoreactive Tg appeared in the fetal rat thyroid gland on the 15th day of gestation, and that both T4 and T3 followed 2 days later (Kawaoi and Tsuneda, 1985). These observations were in good agreement with the finding that the organification of iodine began on the 17th day of gestation in rats (Carpenter, 1959; Remy et al., 1980), when primitive follicles appeared. Rémy et al. (1980) reported that numerous silver deposits of 125I were present over the cytoplasm which bordered the follicular lumina of the thyroid gland of fetal rat at 17 days 11 hours of pregnancy.

The present study investigated the effects of derangement of maternal thyroid function on the fetal development of the thyroid gland in terms of the immunostainability for Tg, T4 and T3 in order to examine the mechanism initiating thyroid hormone production. Thyroidectomy of the pregnant rat failed to alter the date of occurrence of immunoreactive Tg as well as of thyroid hormone, suggesting that a decrease in maternal hormone via the placenta under the present experimental conditions was ineffective. PTU administration caused hyperplasia which was probably due to increased secretion of TSH from the fetal hypophysis, and also degenerative changes in the fetal thyroid gland, where Tg immunoreactivity was preserved. On the other hand, the staining of T4 and T3 was significantly influenced, and ceased almost completely. Not only PTU-induced hypothyroidism of the maternal rat, but also the direct effects of PTU on the fetal thyroid gland via the placenta (As mentioned in a previous study goitrogens could cross the placenta to block fetal thyroid hormone formation (D’Angelo, 1967)), should be taken into consideration when attempting to explain these disturbances of fetal thyroid function.

In view of the inability of TSH to pass through the placenta (Knobil and Josinovich, 1959), the effect of maternal hyperthyroidism induced by exogenous TSH on the fetus should be ascribed to the action of increased release of T4 and T3 from the mother rat. The fetal thyroid gland in this group showed delayed occurrence of T4 and T3 for one day as compared to the control animals, although no prominent atrophy of the gland was demonstrated as in a previous report (Noumura, 1959). An inhibitory effect of maternal hormone on the onset of fetal thyroid hormone synthesis through a negative feedback mechanism was suggested.

Since a stimulative action of TSH on the proliferation and functional maturation of the thyroid epithelium has been observed in an in vitro study by Roger and Dumont, 1983, in which they clarified bi-phasic effects on cultured dog thyroid cells, activating either proliferation or redifferentiation depending on the state of the cells, proof of decreased TSH in the fetal blood of this group could provide evidence that fetal TSH plays a key role in the functional maturation of the thyroid gland.

On the other hand, the ineffectiveness of TSH administration on the appearance of Tg would appear to indicate that the
onset of Tg production and that of either \( T_4 \) or \( T_3 \) are not necessarily sequential.

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**References**


